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July 1964

Chemical Composition of Forages

**in relation to digestibility
by ruminants**

Agricultural Research Service

U.S. DEPARTMENT OF AGRICULTURE

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THE CHEMICAL COMPOSITION OF FORAGES IN RELATION
TO DIGESTIBILITY BY RUMINANTS ^{1/}

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This report serves several purposes. It describes chemical methods used in this Laboratory for the evaluation of forage plants. Some of these methods have been published by others, some are modifications or adaptations of published methods, and some have been developed in this Laboratory. This report also presents in a condensed form the composition of forages as found by these methods, and finally, and this is the primary purpose of this report, these methods have been judged as to their suitability for the evaluation of forage quality.

The evaluation of forages for their nutritive value cannot be accomplished with certainty by chemical analysis but some approximations to nutritive value are possible by this means. The analyses are directed toward those substances that are related to the digestibility of forages by ruminants. The methods have been appraised by relating the results to known digestion coefficients by statistical means.

^{1/} A contribution of the U. S. Regional Pasture Research Laboratory, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, University Park, Pa., in cooperation with the 12 Northeastern Agricultural Experiment Stations.

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^{3/} Grateful acknowledgment is made to many who have contributed to this work. Forage samples were contributed by the Agricultural Experiment Stations of Alabama, Delaware, Maryland, Massachusetts, Missouri, New Hampshire, New Jersey, Ohio, Pennsylvania, Virginia, and West Virginia, by the U. S. Department of Agriculture, and by the Grassland Research Station of the United Kingdom. Special thanks are given to past and present members of the Technical Committee of Regional Project NE 24. Members of the staff of this Laboratory who aided in the chemical analysis, statistical treatment, or preparation of the manuscript are H. P. Binger, P. R. Bono, D. Burdick, Mary L. Denison, J. E. Field, D. S. Frear, G. A. Motter, D. G. Routley, Eleanor Rowland, and E. S. Titlar, Jr.

MATERIALS

Since about 1950, samples of forages with known digestion coefficients, and, in many cases, samples of the feces of animals fed these forages, have been collected from State and Federal experiment stations that had conducted feeding trials. The number of samples of forage exceeds 300. More than half of them were accompanied by samples of feces of one to six animals, so that over 600 samples of feces are available. Knowing the rates of feeding and the quantity of excreta, or even knowing only the digestion coefficient of the dry matter, one may determine the digestion coefficient of many constituents for which analytical procedures are available.

The data donated with the samples varied considerably. In some cases, where complete proximate analyses were made and digestion coefficients of each constituent calculated, all the data were made available. In other cases a part of such data was reported, and in some only the digestion coefficients of dry matter or of organic matter were available. Some data concerning the samples were obtained from published reports. The data also varied to some extent as to reliability. For example, when more than one animal were used in the digestion trials, the average digestion coefficients may be considered more accurate than when only a single animal was used. The data on the digestion coefficients of dry matter were accepted as correct. They could not be repeated. The digestion coefficients of other constituents may be confirmed by repeating the chemical analysis, though their accuracy depends in part on the digestion coefficients of the dry matter. Most of the data reported here were obtained in this Laboratory; when donated data are reported it is so stated, if it is possible to distinguish them.

The forage samples are mainly of dried grasses of species common to the Northeastern United States and of alfalfa. It was the intention when these samples were collected to include only those that had been grown in pure stands and that were fed to animals without supplement, but some were accepted that did not conform to these requirements. Some are mixtures of grass and alfalfa, and in a few cases grain had been added in the feeding trials. Most of the grasses are first cuttings at various stages of growth. It is to be regretted that more aftermath cuttings at different times of the growing season are not available. It is also to be noted that digestion data for the species of grasses and legumes less common to the Northeastern United States are very limited.

METHODS

The samples were ground in an intermediate model Wiley mill to pass through a 40-mesh screen, unless they were of that fineness when received. In many analytical determinations, the fineness of grinding influences the results obtained. However, grinding to a greater fineness than 40-mesh is difficult with most equipment, and it is not done in most laboratories.

Moisture

The loss in weight of a sample treated in a vacuum oven at 60 - 70°C. for 5 hours or overnight was regarded as moisture. All analyses are reported on the moisture-free basis.

Ash

Much of the data on the ash contents was furnished by the State Stations. For total ash, a sample was ignited in a porcelain crucible in a muffle furnace at 550-600°C. for at least 5 hours. The loss in dry matter was regarded as organic matter. Some data are reported in percentage of organic matter rather than of dry matter. Some constituents, such as cellulose and lignin, were determined as the loss of dry matter on ignition. When they were ignited in a crucible with fritted Pyrex disc, they were not heated above 500°C.

Ether Extract

Some of the data on ether extract were furnished by stations that donated the samples. Some samples were analyzed in this Laboratory. In many cases forages on which ether extract data had been received were analyzed again in this Laboratory. Usually a smaller ether extract content was found. For example, 36 forages were received which averaged 3.94 percent ether extract. An average of 3.39 percent was obtained in this Laboratory in those same forages by the following method.

Reagent

Anhydrous ethyl ether, AR, in sealed 1-pound tins.

Procedure

Place a 1-gram sample in a small tin dish and dry overnight in a vacuum oven at 60-70°C. Weigh. Transfer the sample to a filter paper, wrap, and tie with thread. Pack a number of these in the extraction tube of an all-glass Soxhlet apparatus (large size) and place the tube containing the samples in the vacuum oven and again dry. Turn off the heat and allow to partially cool before turning off the vacuum, remove the tube from the oven, and quickly connect the Soxhlet apparatus, which in the meantime had received 1 pound of ether in the 1000-ml. flask. Protect the top of the condenser from moist air with a drying tube. Conduct the extraction overnight at a low heat. Cool, remove the samples, air-dry in a ventilated hood and transfer them from the papers back into the tin boxes and again dry overnight in the vacuum oven. Cool and weigh. The loss in weight is ether extract.

This procedure of extraction was used whenever the residue of the extraction was to be reserved and the soluble matter discarded. For some purposes, as when the ether extract content was not required, it was unnecessary to use anhydrous ether. A mixture of benzene and ethyl alcohol (2.5:1) was used in certain cases, and then it was unnecessary to take precautions against humidity. Lead pencil markings made on the paper were not removed by any solvent used.

Crude Protein

Some of the data on crude protein were furnished by the stations that donated the forage samples. It is assumed that the method used was the Kjeldahl method as described in "Methods of Analysis, A.O.A.C." (2) 4/. All determinations made in this Laboratory were by the micro-Kjeldahl method as described in the same volume.

Alcohol-insoluble Protein

The alcohol-insoluble protein of forages was determined by the micro-Kjeldahl method on the alcohol-insoluble fraction of the forage, which was prepared as described under "Alcohol-insoluble Matter," p. 22.

Crude Fiber

All data on crude fiber were furnished by the stations that donated the forage samples. It is assumed that in each case the method was that described in "Methods of Analysis, A.O.A.C." (2).

Normal-acid Fiber

Normal-acid fiber was proposed by Walker and Hepburn (20) to replace crude fiber. It differs from crude fiber in that the method involves only one boiling with dilute acid and no boiling with sodium hydroxide. Because of, some minor modifications, the procedure is described in detail.

4/ Underscored numbers in parentheses refer to Literature Cited, p. 56.

Procedure

Wrap a 1-gram sample in filter paper, and extract overnight with benzene-alcohol, as described under "Ether Extract." Remove from the paper and place in a 500-ml. wide mouthed Erlenmeyer flask. Introduce 200 ml. boiling 1.0 N H_2SO_4 , with a small amount of wetting agent (triglycolstearate in benzene). Boil under a reflux for 1 hour, and filter hot on an asbestos mat in a Gooch crucible. Wash with hot water, alcohol, and ether. Dry overnight in vacuo at 60-70°C., and weigh. Determine normal-acid fiber (ash-free) as the loss in weight on ignition.

Acetic Acid Fiber

The method for determining acetic acid fiber was proposed by Wilcox and Moxon (21), and involves digestion with pepsin and subsequent boiling of the residue with dilute acetic acid. Difficulty was encountered in wetting the forage sample with the pepsin solution; modifications of the method to overcome this were worked out by E. S. Titlar of this Laboratory.

Reagents

Pepsin, 1 gram of pepsin in 100 ml. 0.1 N HCl .

Acetic acid, 5 percent by weight.

Procedure

Extract a 1-gram sample with benzene-alcohol by the technique described under "Ether Extract." Place in a 300-ml. tall-form beaker. Add about 20 ml. acetone, and mix by swirling to insure complete wetting. Add 50 ml. water and filter through a Gooch crucible containing a circle of hardened filter paper (Whatman #540, 2.4 cm). Wash several times with water. Transfer the residue back to the beaker with the aid of a stream from a wash bottle containing pepsin solution. Remove, rinse, and discard the filter paper. Add in all about 50 ml. pepsin solution. Let the mixture stand overnight at 45°C. Filter again on a disc of filter paper (Whatman #2, cut out with a cork borer) in a Gooch crucible, and wash the residue with water. Transfer to a 500-ml. wide mouthed Erlenmeyer flask with the aid of a stream from a wash bottle containing 5 percent acetic acid. Remove and discard the filter paper. Add about 200 ml. boiling 5 percent acetic acid and boil for one-half hour under a reflux. Filter hot through an asbestos mat in a Gooch crucible, wash with hot water, alcohol, and ether. Dry overnight in vacuo at 60-70°C., and weigh. Determine the acetic acid fiber (ash-free) as the loss in weight on ignition.

Ammonium Oxalate Fiber

The term "ammonium oxalate fiber" is given to that called by Binger et al. (4) "doubly extracted residue." The following procedure determines "ammonium oxalate-insoluble organic matter," and "nonprotein ammonium oxalate-insoluble organic matter," the latter being equal to "total cell wall material" of the above authors except that it is ash-free.

Reagent

0.5 percent aqueous solution of ammonium oxalate.

Procedure

Extract a 1-gram sample with benzene-alcohol by the technique described under "Ether Extract." Place in a 100-ml. centrifuge tube. Add 25 ml. ammonium oxalate solution and place the tube upright in a water bath at 85°C. Attach an air condenser. After 4 hours, centrifuge. Remove the supernatant liquid with the aid of a capillary tube connected with an aspirator and discard. Add an additional 25 ml. oxalate solution, and heat again in the bath overnight. Filter into a glass crucible with fritted disc, and wash the residue with warm water. For the ammonium oxalate-insoluble organic matter (ash-free), weigh and determine the loss in weight on ignition. This quantity is termed here "ammonium oxalate fiber." Treat another 1-gram sample of the forage in the same manner. Instead of igniting the residue, transfer to a Kjeldahl flask and determine its protein content ($N \times 6.25$). Deduct the weight of the protein from the weight of the ammonium oxalate-insoluble organic matter to obtain the amount of nonprotein ammonium oxalate-insoluble organic matter. This entity is termed here "nonprotein ammonium oxalate fiber."

Alcohol-insoluble and Alcohol-soluble Matters

The portion of forage insoluble in 80 percent ethyl alcohol is believed to contain all the organic constituents that are not readily digestible. Conversely, the alcohol-soluble substances are believed to be very highly, or completely digestible. An alcoholic extraction of the forage separates these two fractions. Two substances believed to be highly digestible, fructosan of the grasses and starch of the legumes, are made soluble by the procedure described here and will be found in the alcohol-soluble fraction. The fructosans are readily hydrolyzed by the oxalic acid treatment. Experiments with cornstarch indicated that it is not hydrolyzed by the oxalic acid but that it is solubilized and will not be precipitated on the addition of alcohol. It is assumed that the starch of legume foliage behaves similarly. The same procedure may therefore be used for all forages.

Reagent

A solution of 1 gram oxalic acid in 100 ml. water.

Procedure

Extract a 1-gram sample with ethyl ether or benzene-alcohol by the method described under Ether Extract. Place in a 250-ml. beaker. Add 40 ml. of the oxalic acid solution, bring to boil on an electric hot plate and boil gently 5 minutes. Allow to cool partially, add 160 ml. of 95 percent ethyl alcohol, stir, allow to settle several hours or overnight. Add a small amount of celite (this is optional), stir, and allow to settle. Pour the supernatant liquid through a Gooch crucible containing an asbestos mat. Add about 50 ml. of 80 percent alcohol to the residue in the beaker, warm, and complete the filtration, and wash with warm 80 percent alcohol. Dry at 105°C., or in the vacuum oven at 60-70°C., cool in a desiccator, and weigh. The alcohol-insoluble organic matter, exclusive of fructosan and starch, is the loss in weight on ignition.

If a tared crucible was used and celite not added, the weight of the residue (before ashing) may be assumed to be the alcohol-insoluble dry matter. Such a fraction was used for the determination of alcohol-insoluble protein.

Alcohol-soluble organic matter was calculated by subtraction of both the ether extract and the alcohol-insoluble organic matter from the total organic matter. In this calculation, the ether extract, which was found to have a negligible amount of ash, was assumed to have no ash. Because these forages were dried, some organic compounds may have been altered during the drying. The percentages of soluble matter found in them may not be the same as were present in the original fresh forages, but they may be the same as were present during the digestion trials.

True Cellulose

Cellulose was determined by several methods. The substance which for convenience is called "true cellulose" is determined by the method first proposed by Kürschner and Hanak (9) and also referred to as Crampton and Maynard cellulose (6). The procedure, with slight modifications introduced by Phillips and Smith (13) to obviate the use of a centrifuge, is described as carried out in this Laboratory.

Reagents

A mixture of 650 ml. acetic acid, 80 ml. conc. HNO_3 , and 150 ml. water.

Procedure

Place a 0.5-1.0 gram sample and 25 ml. acetic-nitric reagent in a 300-ml. Florence flask fitted by ground glass connection to a reflux condenser. Boil gently over an electric hot plate for 20 minutes. Remove the condenser, add about 20 ml. ethyl alcohol to the

flask and allow to cool. Pour the contents onto an asbestos mat in a Gooch crucible, with the aid of a stream of 95 percent alcohol from a wash bottle. Wash the residue with warm alcohol and with ether. Dry overnight at 105-110°C., cool in a desiccator containing a good dehydrating agent, and weigh. True cellulose is the loss in weight on ignition at 550-600°C.

Natural Cellulose

The substance which for convenience is called "natural cellulose" is determined by methods described by Norman and Jenkins (12) and by Matrone, Ellis, and Maynard (10). The method described here follows closely that of Matrone et al.

Reagents

- 3 percent sodium sulfite (Na_2SO_3).
- 0.25 N NaOH in 60 percent alcohol.
- 2.5 N H_2SO_4 .
- 20 percent H_2SO_4 (by weight).
- Solution of NaClO (5.25 percent available Cl) (Clorox)

Procedure

Extract 0.5-1.0 gram sample with benzene-alcohol, as described under "Ether Extract." Place in a 250-ml. wide mouthed Erlenmeyer flask. Add 50 ml. sulfite solution, and bring to a boil on a hot plate. Filter, using a filter stick and a layer of celite, and discard the filtrate and all subsequent filtrates and washings by way of the aspirator. Wash out the filter stick by forcing through it backward (with air pressure) 10 ml. of alcoholic NaOH. Stir, neutralize with 1 ml. of 2.5 N H_2SO_4 . Filter again, wash with 20 ml. hot water. Add 43 ml. water and 7 ml. Clorox. Let stand 10 minutes, out of direct sunlight, with occasional stirring with the filter stick. Filter again. Again add 50 ml. sulfite solution, and warm on the steam bath for 10 minutes. Filter. Treat again with alcoholic NaOH, using it to clean out the filter stick, neutralize with H_2SO_4 and filter. This time add 50 ml. cold water, 1.5 ml. Clorox, and 1 ml. 20 percent H_2SO_4 , and let stand 10 minutes. Filter. Repeat the last treatments with sulfite, alcoholic NaOH, and acid Clorox until there are no longer any pink-colored particles, after the addition of the sulfite. Heat the colorless suspension containing sulfite on the steam bath for 10 minutes. Filter and wash with hot water. Transfer the residue with the aid of hot water to a Gooch crucible containing an asbestos mat. Dry at 105-110°C. overnight. Cool in a desiccator containing a good dehydrating agent, and weigh. Natural cellulose is the loss in weight on ignition at 550-600°C.

Total Lignin

Total, or crude lignin, was determined by the method of Ellis, Matrone, and Maynard (7) with some minor modifications.

Reagents

1 percent pepsin in 0.1 N HCl.

3 percent H₂SO₄ by weight (0.632 N).

5 percent H₂SO₄ by weight (1.054 N).

72 percent H₂SO₄ by weight (sp. gr. 1.634 at 20°C.).

Antifoam solution. 1 g. diglycolstearate in 50 ml. alcohol and 50 ml. benzene.

Celite. Ignite beforehand and keep dust free.

Procedure

Extract a 1-gram sample for 30 hours with benzene-alcohol by the technique described under "Ether Extract." Transfer the residue to a 250-ml. wide mouthed Erlenmeyer flask. Add 40 ml. pepsin, cover loosely, and incubate at 38°C. for at least 3 days, shaking occasionally. Filter, using a filter stick and a thin layer of celite. Discard the filtrate and all subsequent filtrates and washings by way of the aspirator. Wash twice with 20-30 ml. portions of water. Return the residue and the celite to the flask by forcing 7-8 ml. of 5 percent H₂SO₄ backward down the filter stick several times with air pressure. Rinse the stick with the same acid from a wash bottle, and add sufficient acid to bring the total volume in the flask to about 125 ml. and add, if necessary to prevent frothing, 2 drops of antifoam. Boil 1 hour under a cold finger condenser; filter hot with the filter stick as before, using a thicker layer of celite; wash three times with hot water, two or more times with 15-20 ml. portions of alcohol or until the filtrate is colorless, and twice with 10-15 ml. portions of ether. Place the flask and filter stick in a warm place to dry, but not on a hot surface. By tapping, gently scraping with a spatula, and brushing with a stiff brush, remove the residue from the stick, and return it to the flask. Add 20 ml. of 72 percent H₂SO₄, stir to remove lumps and to wet each particle with the acid, and place in a cabinet at 20°C. for 2 hours, with stirring at the end of the first hour and again shortly before the second hour. Add 125 ml. cold water, mix by swirling, filter with a filter stick and a thin layer of celite and wash once with 25-30 ml. of cold 3 percent H₂SO₄. Return the residue to the flask as before with 3 percent H₂SO₄ (air pressure); and add enough acid to bring the total to 125 ml. Add 2 drops antifoam, and boil under the condenser 2 hours. Filter hot onto an asbestos mat in a Gooch crucible, and wash thoroughly with hot water. Dry overnight at 105-110°C.; cool in a desiccator, and weigh. Crude lignin is the loss in weight on ignition at 600°C.

Acid-insoluble Lignin

The method for acid-insoluble lignin has been published (17).

Reagents

Benzene-alcohol mixture (2.5:1).

1 percent pepsin in 0.1 N HCl.

72 percent H₂SO₄ by weight (sp. gr. 1.634 at 20°C.).

Conc. HCl.

Procedure

Place a 1-gram sample in a tall 200-ml. beaker. Add 30-40 ml. benzene-alcohol. Boil gently on a steam bath for several hours or allow to stand overnight and then boil for a short time. Pour into a Gooch-type crucible (low-form) with fritted Pyrex disc, size 30 C, containing an asbestos mat, and allow to filter slowly with light suction. Rinse the residue 2-3 times with benzene-alcohol, 3 times with acetone, and 3 times with water. Transfer as much solid material as possible, including the asbestos, back to the beaker, and add pepsin solution. This can be done easily with a rod and with the pepsin solution in a wash bottle. Use about 40 ml. pepsin solution in all. Allow to incubate at 45°C. for 20-24 hours; mix occasionally during the first few hours. Then transfer the mixture quantitatively into the same crucible, wash several times with water, pour through it about 25 ml. HCl, previously cooled to about 15°C., adjusting the flow so that the HCl is in contact with the sample for about 2 minutes. Wash with water, alcohol, and ether, and allow to air-dry. Add sufficient 72 percent H₂SO₄, previously cooled to about 15°C., to cover the contents of the crucible. Stir with a 3-inch flattened glass rod which is allowed to remain for the time in the crucible. Be sure all particles come in contact with the acid, and break up any lumps. Place the crucible in a 50-ml. beaker for support and for receiving the acid which percolates through slowly and is to be discarded. Maintain the temperature as close to 20°C. as convenient. Stir occasionally and add fresh acid as necessary to keep the mixture fluid. At the end of about 3 hours return the crucible to the suction flask and remove the acid, without dilution, by filtration. Wash the residue thoroughly with hot water to remove all traces of acid. Dry overnight at 105-110°C. and weigh. Acid-insoluble lignin is the loss in weight on ignition at 500°C.

Detergent Fiber and Detergent Lignin

The methods for determining detergent fiber and detergent lignin in the same sample were proposed by Van Soest (19). Unlike the method for the determination of crude fiber the preliminary extraction of the sample to remove fatty materials is unnecessary and only one heating, that with acid

detergent, is necessary. The lignin determination is performed on the isolated fiber fraction. Modifications are made here in the size sample and in the volume of reagents. Another change is that the fiber was determined on the ash-free basis.

Reagent

2 percent acid detergent (hexadecyltrimethylammonium bromide) in 1.0 N H_2SO_4 .

Procedure

Weigh a 1-gram sample into a 250-ml. wide mouthed flask. Add 50 ml. of a solution of acid detergent and heat over a hot plate at low heat so that boiling commences in 10-12 minutes. Continue boiling at higher heat for 60 minutes. Filter through a sintered glass crucible with light suction. Wash with hot water and several times with acetone. Dry and weigh. Determine the weight of fiber as the loss in weight on ignition at 500°C . For detergent lignin, prepare the detergent fiber as above and to it in the crucible (without ashing) add a small quantity of asbestos and then some 72 percent H_2SO_4 ; continue as described under the method for acid-insoluble lignin. The method for detergent lignin is essentially an improved one for acid-insoluble lignin.

RESULTS

Table 1 gives a summary of the composition of the forages and of the digestion coefficients of some of the constituents. Because of the limited size of some of the samples and because of the labor involved, not all samples were analyzed for every constituent. The number of samples analyzed for the purpose of evaluating a particular method varied; the samples selected for a particular study depended, in part, on the quantity of material on hand and on the desire to cover a range in quality.

Moisture

As all the forage samples were dry when received, the original moisture content of the standing crop was unknown and probably had not been determined in most cases. The dried ground samples in equilibrium with the air contained 5-10 percent moisture which could be removed by oven-drying. All forage constituents are expressed on the oven-dry basis.

Table 1.--Summary of chemical composition and of some digestion coefficients (D/C) of forages. Percentages are on the basis of oven-dry weights.

Constituent	Grass				Alfalfa			
	Composition		Digestion coeff.		Composition		Digestion coeff.	
	No. tests	Av. %	No. tests	Av. D/C	No. tests	Av. %	No. tests	Av. D/C
Dry matter	---	---	101	69.4	---	---	54	61.1
Organic matter	86	92.9	125	70.4	43	91.3	36	62.5
Ether extract	60	3.1	54	43.8	41	2.0	10	29.4
Crude protein	101	14.7	76	64.9	54	18.6	50	72.0
Soluble protein	42	^a 25.8	---	---	9	^a 31.4	---	---
Crude fiber	65	31.6	38	71.9	20	31.5	16	49.2
Normal-acid fiber	25	40.8	---	---	9	41.3	---	---
Acetic acid fiber	20	56.0	---	---	9	48.3	---	---
Detergent fiber	36	33.6	---	---	13	35.2	---	---
Oxalate fiber	21	66.5	---	---	9	57.0	---	---
Nonprotein oxalate fiber	21	54.7	---	---	9	44.0	---	---
Alcohol-insoluble matter	67	^b 77.4	43	63.0	25	^b 75.1	18	51.2
Nonprotein alcohol-insoluble matter	67	^b 67.5	44	64.1	25	^b 62.1	15	45.3
Alcohol-soluble matter	64	^b 19.7	---	---	24	^b 22.4	---	---
True cellulose	46	30.7	46	75.4	20	29.4	13	61.9
Natural cellulose	24	39.1	24	75.4	1	26.1	1	61.6
Total lignin	44	6.9	---	---	20	9.6	---	---
Acid-insoluble lignin	101	5.0	---	---	27	9.8	---	---
Detergent lignin	36	4.2	---	---	13	7.2	---	---

^a In percentage of total crude protein.

^b In percentage of organic matter.

Ash

The ash content of 86 grasses ranged from 4.5 to 10.4 percent, with an average of 7.1. In 43 alfalfa samples it ranged from 5.3 to 12.4, with an average of 8.7. Seven mixtures averaged 7.5 percent ash.

Ether Extract

The ether extract content of 60 grasses ranged from 1.5 to 5.2 with an average of 3.1 percent. The digestion coefficients of the ether extracts showed considerable variation, ranging from 3.3 to 62.5, with an average of 43.8. The variation among animals as well as the variation among samples of otherwise similar composition indicated that digestion data of ether extract are less reliable than those of other constituents. Alfalfa contained less ether extract than the grasses; 41 samples averaged 2.0 percent and the digestion coefficient of 10 samples averaged 29.4. No further use was made of these data.

Protein

The crude protein content is widely used as a criterion of quality in forages. Apart from protein as an essential nutrient, its quantity is reported to be correlated positively with dry matter digestibility and with its own digestibility. About half of the protein data was obtained from the stations that supplied the samples and about half, including all of the insoluble protein data, was obtained in this Laboratory.

Relation of crude protein to the digestibility of dry matter.--In 101 samples of grass (bromegrass 32, orchardgrass 22, ryegrass 22, Kentucky bluegrass 12, timothy 9, and sudangrass 4), the ranges, averages, and statistical values were:

Crude protein in percent of dry matter (X), 5.3-30.9; Av. 14.7.

Digestion coefficient of dry matter (Y), 48.1-81.8; Av. 69.4.

$r = +0.61^{**} \frac{5/}{}$; $Y = 63.5 + 0.40 X$; S.E. = ± 6.1 ; CV = 8.7.

From these results it is apparent that the correlation coefficient (r) between the percent of crude protein and the digestion coefficient of dry matter is positive and of high significance. The digestion coefficient of the dry matter may be predicted from the percent of protein with a standard error (S.E.) of

5/ *Indicates a significance of 5 percent and ** of 1 percent.

6.1 units and a coefficient of variation (CV) of 8.7. In 54 samples of alfalfa the data were:

Crude protein in percent of dry matter (X), 13.8-24.4; Av. 18.6.

Digestion coefficient of dry matter (Y), 52.6-74.3; Av. 61.1.

$r = +0.40^{**}$; $Y = 46.2 + 0.80 X$; S.E. = ± 3.9 ; CV = 6.3.

In 10 samples of mixed grass and alfalfa the data were:

Crude protein in percent of dry matter (X), 7.9-17.0; Av. 12.3.

Digestion coefficient of dry matter (Y), 51.0-73.3; Av. 62.6.

$r = +0.57$; $Y = 47.2 + 1.25 X$; S.E. = ± 5.0 ; CV = 7.9.

Of these 10 samples the alfalfa content is known in only 7 and ranged from 3 to 45 percent of the total. Except for the fact that the two samples with the highest alfalfa content, 37 and 45 percent, were the highest in percent protein, 14.6 and 17.0, there were no significant relationships between the alfalfa content and the percent of protein of the mixture or the digestion coefficient of dry matter. When the three populations were combined to make a total of 165 samples of forage:

$r = +0.24^{**}$; $Y = 60.7 + 0.35 X$; S.E. = ± 7.1 ; CV = 10.7.

Figure 1 is a scatter diagram of these results. While protein is a valuable constituent in its own right, its use as a predictor of digestibility of total dry matter seems, in these samples, to be limited because of the divergence between the predicted dry matter digestibility and that found by conventional means.

Relation of crude protein to its own digestibility.--In 76 samples of grass (bromegrass 36, orchardgrass 18, timothy 9, sudangrass 4, Kentucky bluegrass 5, and reed canarygrass 4), the data were:

Crude protein in percent of dry matter (X), 5.3-25.8; Av. 13.2.

Digestion coefficient ^{6/} of crude protein (Y), 34.6-79.9, Av. 64.9.

$r = +0.85^{**}$; $Y = 42.7 + 1.70 X$; S.E. = ± 5.2 ; CV = 7.9.

In 50 samples of alfalfa the data were:

Crude protein in percent of dry matter (X), 13.8-23.9; Av. 18.5.

Digestion coefficient of crude protein (Y), 51.9-79.7; Av. 72.0.

$r = +0.61^{**}$; $Y = 48.5 + 1.28 X$; S.E. = ± 3.3 ; CV = 4.6.

In 10 samples of mixed grass and legume, the data were:

Crude protein in percent dry matter (X), 7.9-17.0; Av. 12.3.

Digestion coefficient of crude protein (Y), 57.2-73.8; Av. 64.0.

$r = +0.25$; $Y = 50.4 + 1.11 X$; S.E. = ± 4.2 ; CV = 6.5.

^{6/} Digestion coefficients of any form of protein are assumed to be "apparent", whether so stated or not.

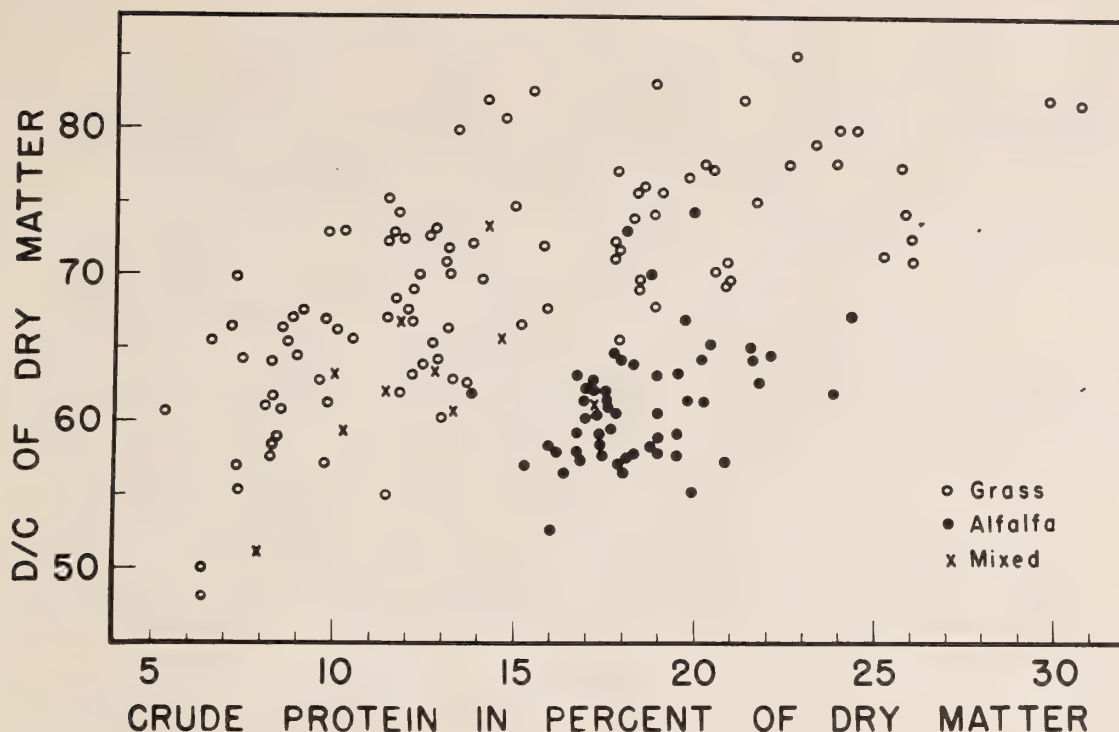


Figure 1.--Relation between crude protein in percentage of dry matter and the digestion coefficient of dry matter. For all samples, $r = +0.24$.

When the three populations were combined to make a total of 136 samples--grass, alfalfa, and mixtures:

$$r = +0.86^{**}; Y = 44.4 + 1.54 X; S.E. = \pm 4.5; CV = 6.7.$$

The percentage of crude protein will predict the apparent digestion coefficient of crude protein with more accuracy than it will predict the digestion coefficient of dry matter. This may be seen by comparing the coefficients of variation for the corresponding classes of forage.

Some writers have expressed the relationship between protein and its own digestibility in a slightly different way, as between the percent of crude protein and the percent of digestible protein. On the same 76 samples of grass, the data were:

Crude protein in percent of dry matter (X), 5.3-25.8; Av. 13.2.

Digestible protein in percent of dry matter (Y), 2.2-20.6; Av. 9.0.

$$r = +0.995^{**}; Y = -2.84 + 0.90 X; S.E. = \pm 0.42; CV = 4.6.$$

In the same 50 samples of alfalfa mentioned above, the data were:

Crude protein in percent of dry matter (X), 13.8-23.9; Av. 18.5.

Digestible protein in percent of dry matter (Y), 8.5-17.8; Av. 13.3.

$$r = +0.95^{**}; Y = -4.19 + 0.95 X; S.E. = \pm 0.59; CV = 4.4.$$

In the 10 samples of mixed grass and alfalfa, the data were:

Crude protein in percent of dry matter (X), 7.9-17.0; Av. 12.3.

Digestible protein in percent of dry matter (Y), 4.6-11.6; Av. 7.7.

$r = +0.97^{**}$; $Y = -1.59 + 0.78 X$; S.E. = ± 0.54 ; CV = 7.1.

When the three populations were combined to a total of 136 samples:

$r = +0.99^{**}$; $Y = -2.73 + 0.88 X$; S.E. = ± 0.52 ; CV = 5.0.

A scatter diagram of these data appears in Figure 2. When the relationship between crude protein and its digestibility was expressed in this way, very high correlation coefficients were obtained but there still remained a considerable coefficient of variation of the prediction. While this variation, expressed as the standard error, is large in relation to the percentage of digestible protein it is not large in relation to total digestible matter in the prediction of which, and in conjunction with other data, it will be used later. The percentage of crude protein will, therefore, predict the percentage of digestible protein more accurately than it will predict the digestion coefficients of either crude protein or total dry matter. Its prediction of digestible dry matter is subject to considerable error.

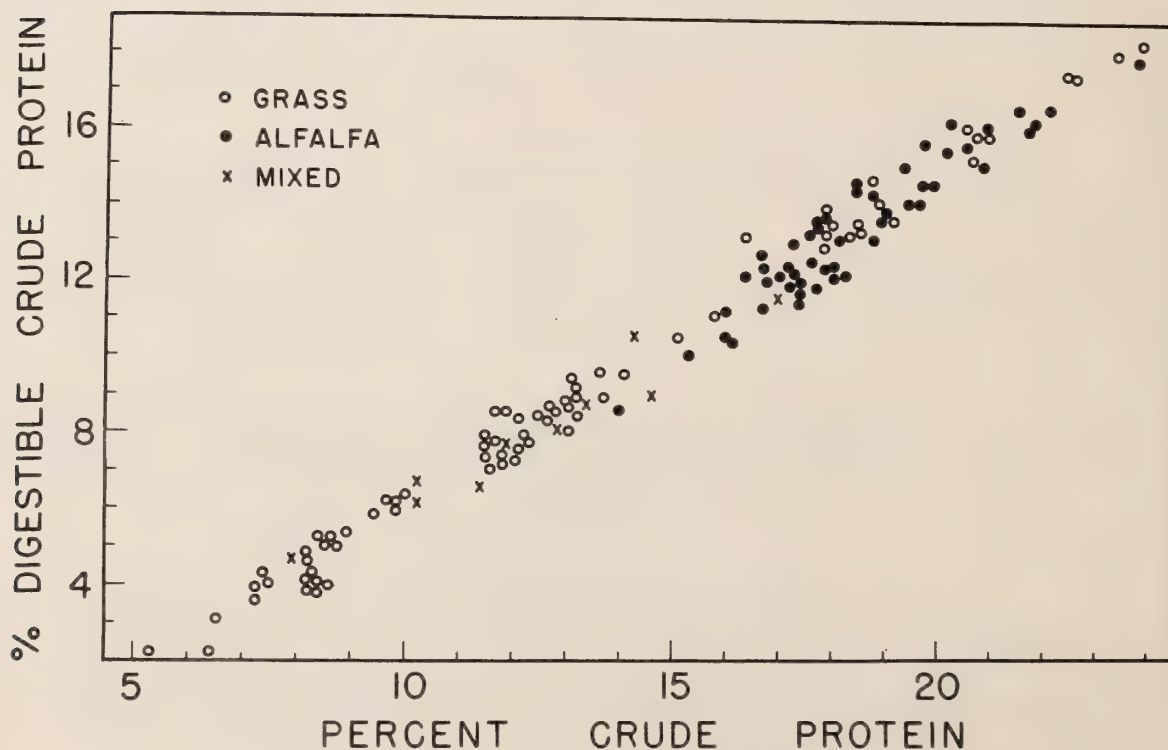


Figure 2.--Relation between crude protein and digestible crude protein, both in percentage of dry matter. For all samples, $r = +0.99^{**}$.

Crude Fiber

Crude fiber is perhaps the most widely used criterion of quality in forages, from the energy standpoint. The quantity of crude fiber is intended to measure the quantity of the less digestible part of the forage. It is generally considered that the greater the crude fiber the lower is the digestibility. Because the crude fiber data examined here were obtained from the stations which furnished the samples of forage, some variability may be owing to the fact that the analyses were made in different laboratories.

Relation of crude fiber to the digestibility of dry matter.--In 65 samples of grass (bromegrass 26, orchardgrass 15, timothy 13, Kentucky bluegrass 5, reed canarygrass 4, and unspecified mixed grass 2), the data were:

Crude fiber in percent of dry matter (X), 20.8-41.3; Av. 31.6.

Digestion coefficient of dry matter (Y), 50.0-77.5; Av. 67.8.

$r = -0.65^{**}$; $Y = 93.4 - 0.81 X$; S.E. = ± 4.1 ; CV = 6.0.

A scatter diagram, indicating the grass species, appears in Figure 3.

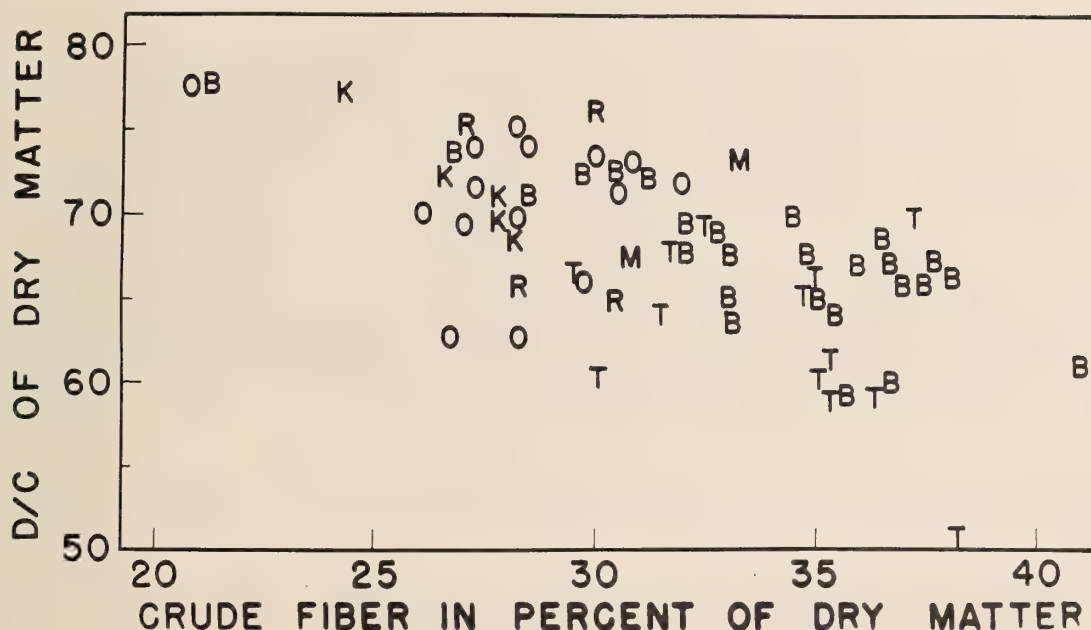


Figure 3.--Relation between crude fiber in percent of dry matter and the digestion coefficient of dry matter, for grasses, by species. The abbreviations are B for bromegrass, O orchardgrass, T timothy, K Kentucky bluegrass, R reed canarygrass, and M mixed grasses. For all grasses, $r = -0.65^{**}$.

In 20 samples of alfalfa, the data were:

Crude fiber in percent of dry matter (X), 24.1-45.6; Av. 31.5.

Digestion coefficient of dry matter (Y), 56.8-70.1; Av. 61.6.

$$r = -0.54^*; \quad Y = 73.6 - 0.38 X; \quad S.E. = \pm 3.5; \quad CV = 5.7.$$

Thirteen samples were reported as mixtures of grass and alfalfa. The data were:

Crude fiber in percent of dry matter (X), 27.9-35.9; Av. 32.6.

Digestion coefficient of dry matter (Y), 60.6-71.5; Av. 65.1.

$$r = +0.49; \quad Y = 46.1 + 0.58 X; \quad S.E. = \pm 2.9; \quad CV = 4.5.$$

It is to be noted that the last correlation coefficient, though without significance, is positive, which is contrary to those with either grass alone or alfalfa alone. In these mixtures the alfalfa content ranged from 5 to 65 percent, with an average of 26. There was a positive correlation, $r = +0.62^*$, between the percentage of alfalfa in the mixture and the percentage of crude fiber but only a negligible correlation, $r = +0.14$, between the percentage of alfalfa and the digestion coefficient of dry matter. The mixtures with a greater proportion of alfalfa tended to be higher in crude fiber, but they were not different in digestibility of dry matter. When the three populations were combined to make a total of 98 samples:

$$r = -0.49^{**}; \quad Y = 84.9 - 0.59 X; \quad S.E. = \pm 4.8; \quad CV = 7.3.$$

The regression lines for crude fiber and digestibility of dry matter are given in Figure 4. Higher fiber contents are associated with lower digestibility in both grasses and alfalfa. However, the mathematical relationships in the two types of forage are not the same. If the equation for grass were used for alfalfa, the predicted digestion coefficient would be very high, particularly at low fiber levels. Moreover, where grass and alfalfa have been grown in association, the crude fiber percentages and the digestion coefficients of the mixtures are correlated positively. Higher fiber content is associated with higher digestibility but also, in this limited number of samples, with a higher proportion of alfalfa in the mixture. In general these data show that crude fiber is a better predictor of digestibility in pure stands of grass or alfalfa than in mixtures.

Relation of crude fiber to its own digestibility.--Criticisms that have been made of crude fiber as a criterion of forage digestibility are that it is not a single or uniform substance and that its composition varies from sample to sample. These would not be serious criticisms if there were a relationship between the quantity of fiber and its own digestibility. In forages of high fiber content the fiber tends to have low digestibility.

In 38 samples of grass (bromegrass 20, orchardgrass 7, timothy 7, reed canarygrass 3, and Kentucky bluegrass 1), the data were:

Crude fiber in percent of dry matter (X), 20.8-41.3; Av. 32.5.

Digestion coefficient of crude fiber (Y), 51.5-85.6; Av. 71.9.

$$r = -0.61^{**}; \quad Y = 100.9 - 0.90 X; \quad S.E. = \pm 5.6; \quad CV = 7.8.$$

In 16 samples of alfalfa, the data were:

Crude fiber in percent of dry matter (X); 25.1-45.6; Av. 30.9.

Digestion coefficient of crude fiber (Y), 39.4-62.0; Av. 49.2.

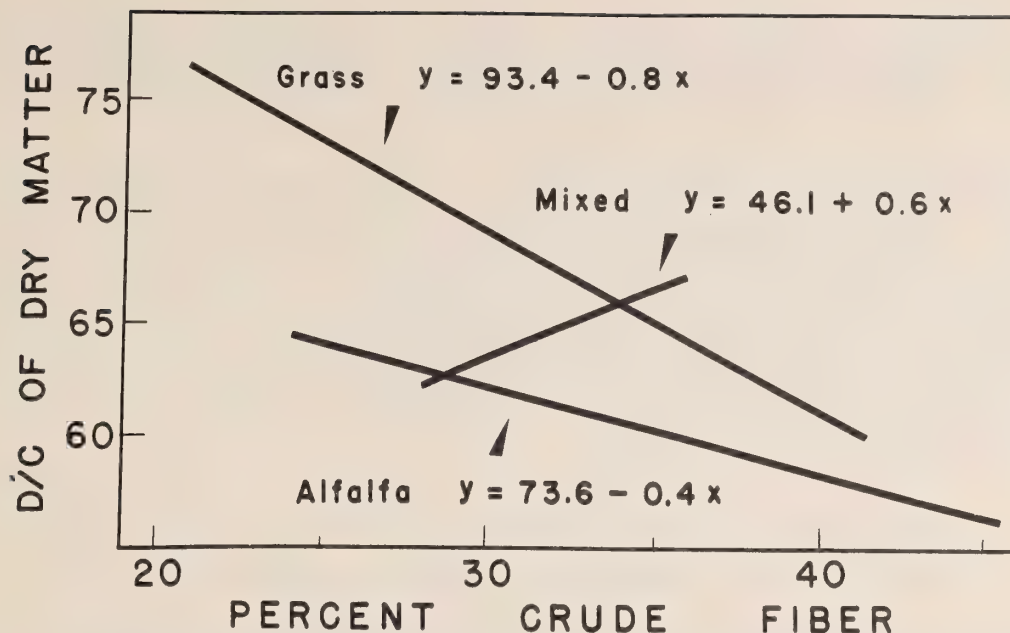


Figure 4.--Regression lines of crude fiber in percentage of dry matter on the digestion coefficient of dry matter for grasses, for alfalfa, and for samples of grass and alfalfa mixed.

$$r = +0.19; Y = 41.0 + 0.27 X; S.E. = \pm 7.5; CV = 15.1.$$

When the two populations were combined to make a total of 54 samples:

$$r = -0.08; Y = 71.4 - 0.19 X; S.E. = 12.5; CV = 19.0.$$

From these data it appears that the value of the crude fiber estimation in grasses is that its percentage is correlated significantly with its own digestibility, a relationship that does not hold in alfalfa. In spite of this significant correlation the error of the prediction is rather high, 5.6.

Normal-Acid Fiber

Relation of normal-acid fiber to the digestibility of dry matter.--In 25 samples of grass (orchardgrass 9, ryegrass 6, bromegrass 4, timothy 4, and Kentucky bluegrass 2), the data were:

Normal-acid fiber in percent of dry matter (X), 29.9-49.0; Av. 40.8.

Digestion coefficient of dry matter (Y), 43.2-82.5; Av. 69.2.

$$r = -0.79^{**}; Y = 124.4 - 1.35 X; S.E. = \pm 5.7; CV = 8.5.$$

In 9 samples of alfalfa the data were:

Normal-acid fiber in percent of dry matter (X), 35.2-47.0; Av. 41.3.

Digestion coefficient of dry matter (Y), 51.4-64.2; Av. 59.0.

$$r = -0.91^{**}; \quad Y = 100.4 - 1.00 X; \quad S.E. = \pm 1.6; \quad CV = 2.7.$$

When the two populations were combined to make a total of 34 samples:

$$r = -0.71^{**}; \quad Y = 121.0 - 1.33 X; \quad S.E. = \pm 6.5; \quad CV = 9.8.$$

The number of samples chosen for this study is smaller than that used for crude fiber, but it covers a similar range in digestion coefficients. It is to be noted that the percentages of normal-acid fiber are higher than those of crude fiber because of the less drastic conditions to which the sample was subjected during the analysis. In grass and alfalfa of the same normal-acid fiber content the alfalfa has, on the average, lower dry matter digestibility. From the CV's, particularly those for grass and for the combined population, it appears that normal-acid fiber has no advantage over crude fiber as a criterion of digestibility.

Acetic Acid Fiber

Relation of acetic acid fiber to the digestibility of dry matter.--In 20 samples of grass (orchardgrass 8, timothy 4, brome grass 4, ryegrass 2, and Kentucky bluegrass 2), the data were:

Acetic acid fiber in percent of dry weight (X), 39.3-71.2; Av. 56.0.

Digestion coefficient of dry matter (Y), 43.2-85.2; Av. 69.8.

$$r = -0.86^{**}; \quad Y = 116.2 - 0.83 X; \quad S.E. = \pm 4.6; \quad CV = 6.6.$$

In 9 samples of alfalfa, the data were:

Acetic acid fiber in percent of dry matter (X), 37.0-58.9; Av. 48.6.

Digestion coefficient of dry matter (Y), 51.4-71.4; Av. 59.8.

$$r = -0.95^{**}; \quad Y = 99.7 - 0.82 X; \quad S.E. = \pm 1.8; \quad CV = 3.0.$$

When the two populations were combined to make a total of 29 samples:

$$r = -0.70^{**}; \quad Y = 105.1 - 0.71 X; \quad S.E. = \pm 8.3; \quad CV = 12.5.$$

The percentages of acetic acid fiber are greater than those of normal-acid fiber and crude fiber. In the limited number of samples, the correlation coefficients between the fiber and dry matter digestibility are high in both grasses and alfalfa considered separately and the CV's are not high. However, the regression lines for grass and alfalfa are far apart, and a suitable regression does not exist for both types of forage.

Detergent Fiber

In this very rapid fiber method the weight of the fiber was related to the digestibility of the forage.

Relation of detergent fiber to the digestibility of dry matter.--In 36 samples of grass (orchardgrass 10, brome grass 6, reed canarygrass 5, Kentucky bluegrass 4, timothy 5, and ryegrass 6), the data were:

Detergent fiber in percent of dry matter (X), 18.8-44.3; Av. 33.6.

Digestion coefficient of dry matter (Y), 43.2-82.5; Av. 68.0.

$r = -0.78^{**}$; $Y = 106.4 - 1.14 X$; S.E. = ± 5.5 ; CV = 8.1.

In 13 samples of alfalfa, the data were:

Detergent fiber in percent of dry matter (X), 26.1-41.6; Av. 35.2.

Digestion coefficient of dry matter (Y), 52.6-74.3; Av. 61.3.

$r = -0.83^{**}$; $Y = 96.1 - 0.99 X$; S.E. = ± 3.0 ; CV = 4.9.

In 7 samples of mixed grass and alfalfa, the data were:

Detergent fiber in percent of dry matter (X), 32.4-39.0; Av. 35.6.

Digestion coefficient of dry matter (Y), 60.5-66.3; Av. 62.9.

$r = +0.87^{**}$; $Y = 33.0 + 0.84 X$; S.E. = ± 1.2 ; CV = 1.9.

When the three populations were combined to make a total of 56 samples:

$r = -0.76^{**}$; $Y = 104.6 - 1.14 X$; S.E. = ± 5.3 ; CV = 8.1.

In this limited study, detergent fiber does not appear to be an improvement over crude fiber as a predictor of dry matter digestibility. The regression equations for grass and alfalfa are quite different from each other. As with crude fiber there is a positive correlation between the two quantities in forage mixtures.

Ammonium Oxalate Fiber

Relation of ammonium oxalate fiber to digestibility of dry matter.--In 21 samples of grass (orchardgrass 8, brome grass 6, timothy 2, Kentucky bluegrass 2, ryegrass 2, and reed canarygrass 1), the data were:

Ammonium oxalate fiber in percent of dry matter (X), 52.2-75.8; Av. 66.5.

Digestion coefficient of dry matter (Y), 46.9-82.5; Av. 69.8.

$r = -0.83^{**}$; $Y = 159.3 - 1.35 X$; S.E. = ± 5.1 ; CV = 7.3.

In 9 samples of alfalfa, the data were:

Ammonium oxalate fiber in percent of dry matter (X), 51.1-63.5; Av. 57.0.

Digestion coefficient of dry matter (Y), 56.9-65.0; Av. 61.7.

$r = -0.62$; $Y = 87.3 - 0.45 X$; S.E. = ± 2.1 ; CV = 3.4.

When the two populations were combined with 3 additional samples, which were mixtures, to make a total of 33 samples:

$r = -0.26$; $Y = 86.4 - 0.30 X$; S.E. = ± 7.8 ; CV = 12.2.

This again illustrates the difficulty of applying one method to both grasses and legumes. The results show a significant correlation in grasses but not in alfalfa. However, the error is less in alfalfa, though the number of samples may be too small for a valid comparison. When both classes were thrown together and three additional samples included, an insignificant correlation of -0.26 was obtained.

Relation of nonprotein ammonium oxalate fiber to digestibility of dry matter.--Ammonium oxalate residues, similar to those above but without ashing, were analyzed for total nitrogen, and the weights of the nonprotein residues were calculated. Such a residue would be equivalent to the "total cell wall material" as reported by Binger et al. (4), except that it is on the ash-free basis. In the same 21 grasses the data were:

Nonprotein oxalate fiber in percent of dry matter (X), 38.9-71.3;
Av. 54.7.

Digestion coefficient of dry matter (Y), 46.9-82.5; Av. 69.8.

$r = -0.86^{**}$; $Y = 111.3 - 0.76 X$; S.E. = ± 4.5 ; CV = 6.5.

In the same 9 samples of alfalfa, as above, the data were:

Nonprotein oxalate fiber in percent of dry matter (X), 35.4-51.4;
Av. 44.0.

Digestion coefficient of dry matter (Y), 56.9-65.0; Av. 61.7.

$r = -0.70^{*}$; $Y = 77.3 - 0.36 X$; S.E. = ± 2.0 ; CV = 3.1.

Again, the grasses and alfalfa fall into different patterns. When the two populations were combined with 3 samples of mixed grass and alfalfa to make a total of 33 samples:

$r = -0.41^{*}$; $Y = 84.7 - 0.34 X$; S.E. = ± 7.3 ; CV = 10.9.

Alcohol-insoluble Matter

The alcohol-insoluble matter probably includes all the cell wall material together with much of the protein and other substances insoluble in 80 percent alcohol. It differs from crude fiber in being much greater in quantity and including much more protein, cellulose, hemicellulose, and lignin. Because it represents such a large proportion of the organic matter of the forage it is here presented in percentage of the total organic matter instead of dry matter.

Relation of alcohol-insoluble matter to the digestibility of organic matter.--In 67 samples of grass (bromegrass 31, orchardgrass 18, timothy 10, Kentucky bluegrass 4, and sudangrass 4), the data were:

Alcohol-insoluble matter in percent of organic matter (X), 70.2-86.4;
Av. 77.4.

Digestion coefficient of organic matter (Y), 55.2-80.1; Av. 68.0.

$r = -0.63^{**}$; $Y = 167.0 - 1.28 X$; S.E. = ± 4.9 ; CV = 7.2.

In 25 samples of alfalfa, the data were:

Alcohol-insoluble matter in percent of organic matter (X), 67.9-84.1;
Av. 75.1.

Digestion coefficient of organic matter (Y), 53.4-69.1; Av. 61.4.

$r = -0.67^{**}$; $Y = 105.9 - 0.59 X$; S.E. = ± 3.1 ; CV = 5.0.

When the two populations were combined to make a total of 92 samples:

$r = -0.39^{**}$; $Y = 118.0 - 0.68 X$; S.E. = ± 6.0 ; CV = 9.0.

The use of the alcohol-insoluble matter as an index of organic matter digestibility involves fairly large errors. This fraction may be broken down further to consider its carbohydrate portion only, exclusive of the protein.

Relation of nonprotein alcohol-insoluble matter to the digestibility of organic matter.--Nitrogen was determined on the alcohol-insoluble matter and the percentage of nonprotein alcohol-insoluble matter was calculated. In the same 67 samples of grass mentioned above, the data were:

Nonprotein alcohol-insoluble matter in percent of organic matter (X);
49.1 - 78.3; Av. 67.5.

Digestion coefficient of organic matter (Y), 55.2-80.1; Av. 68.0.

$r = -0.76^{**}$; $Y = 121.3 - 0.79 X$; S.E. = ± 4.1 ; CV = 6.1.

In the same 25 samples of alfalfa mentioned above, the data were:

Nonprotein alcohol-insoluble matter in percent of organic matter (X),
54.2-74.9; Av. 62.1.

Digestion coefficient of organic matter (Y), 53.4-69.1; Av. 61.4.

$r = -0.73^{**}$; $Y = 99.6 - 0.61 X$; S.E. = ± 2.8 ; CV = 4.6.

The errors are moderate, in comparison with those found by various fiber methods, but the two regression lines are not close to each other. If the two populations were combined to make a total of 92 samples:

$r = -0.44^{**}$; $Y = 96.2 - 0.45 X$; S.E. = ± 5.8 ; CV = 8.9.

The weight of the nonprotein alcohol-insoluble portion is more closely correlated with the digestion coefficient of dry matter than is the entire alcohol-insoluble fraction, and the errors of prediction are less but only slightly so.

Relation of alcohol-insoluble matter to its own digestibility.--A relation may also be calculated between the quantity of alcohol-insoluble matter and its own digestion coefficient, as was done with crude fiber. The digestion coefficient of the alcohol-insoluble matter was calculated in the following manner. It is assumed that the alcohol-soluble portion of the forage (excluding ether extract) is completely digestible (see next section). If this is so, the entire organic matter of the feces (less ether extract) must have been derived from the alcohol-insoluble portion of the forage. Therefore the digestion coefficient of the alcohol-insoluble portion may be calculated from the ether-insoluble, alcohol-insoluble portion of the forage and the ether-insoluble portion of the feces, both ash-free.

In 43 samples of grass (orchardgrass 21, timothy 7, bromegrass 7, Kentucky bluegrass 4, and sudangrass 4), the data were:

Alcohol-insoluble matter in percent of organic matter (X), 69.8-85.6;
Av. 76.2.

Digestion coefficient of the insoluble matter (Y), 41.6-77.2; Av. 63.0.
 $r = -0.58^{**}$; $Y = 163.2 - 1.31 X$; S.E. = ± 6.3 ; CV = 10.0.

In 18 samples of alfalfa, the data were:

Insoluble matter in percent of organic matter (X), 68.6-84.1; Av. 75.9.

Digestion coefficient of alcohol-insoluble matter (Y), 46.6-57.5;
Av. 51.2.

$r = -0.34$; $Y = 72.0 - 0.27 X$; S.E. ± 3.5 ; CV = 6.8.

When the two populations were combined to make a total of 61 samples:

$r = -0.32^*$; $Y = 115.3 - 0.73 X$; S.E. = ± 8.3 ; CV = 14.0.

The weight of the alcohol-insoluble portion is not as clearly related to its own digestibility as is the weight of crude fiber to its digestibility. The nonprotein portion of the insoluble matter will also be considered.

Relation of nonprotein alcohol-insoluble matter to its own digestibility.

--In 44 grass samples (orchardgrass 18, timothy 9, bromegrass 8, Kentucky bluegrass 5, and sudangrass 4), the data were:

Nonprotein alcohol-insoluble matter in percent of organic matter (X),
49.1-78.3; Av. 66.0.

Digestion coefficient of nonprotein alcohol-insoluble matter (Y),
43.8-77.8; Av. 64.1.

$r = -0.76^{**}$; $Y = 122.6 - 0.89 X$; S.E. = ± 5.0 ; CV = 7.8.

In 15 samples of alfalfa, the data were:

Nonprotein alcohol-insoluble matter in percent of organic matter (X),
57.3-74.9; Av. 62.6.

Digestion coefficient of nonprotein alcohol-insoluble matter (Y),
36.1-55.1; Av. 45.3.

$r = -0.44$; $Y = 78.4 - 0.53 X$; S.E. = ± 5.8 ; CV = 12.8.

When the two populations were combined to make a total of 59 samples:

$r = -0.27^*$; $Y = 89.7 - 0.47 X$; S.E. = ± 10.1 ; CV = 17.0.

The errors of all these predictions are quite high. It does not seem that the alcohol-insoluble matter or the carbohydrate part of it can be used alone as a simple predictor of digestibility, particularly in mixtures containing alfalfa.

Alcohol-soluble Matter

The alcohol-soluble matter was calculated by subtracting both the ether extract and the alcohol-insoluble matter from the total organic matter, all on the ash-free basis.

Relation of alcohol-soluble matter to the digestibility of organic matter.--In 64 samples of grass (bromegrass 29, orchardgrass 18, timothy 8, Kentucky bluegrass 5, and sudangrass 4), the data were:

Alcohol-soluble matter in percent of organic matter (X), 10.9-25.6;
Av. 19.7.

Digestion coefficient of organic matter (Y), 51.2-80.1; Av. 68.0.

$r = +0.61^{**}$; $Y = 38.9 + 1.48 X$; S.E. = ± 5.0 ; CV = 7.4.

In 24 samples of alfalfa, the data were:

Alcohol-soluble matter in percent of organic matter (X), 14.1-29.4;
Av. 22.4.

Digestion coefficient of organic matter (Y), 53.4-66.2; Av. 60.9.

$r = +0.66^{**}$; $Y = 49.1 + 0.53 X$; S.E. = ± 2.7 ; CV = 4.4.

When the two populations were combined with 4 mixtures to make a total of 92 samples:

$r = +0.28^{**}$; $Y = 55.7 + 0.51 X$; S.E. = ± 6.2 ; CV = 9.4.

The soluble matter of the forage is correlated positively with the digestibility of total organic matter. This relationship is not close enough for the purpose of using the percentage of soluble matter as a predictor of digestibility. The alcohol-soluble matter is a fraction which may have been modified by circumstances of harvesting and preservation as well as by environmental and cultural conditions previous to harvesting. It is therefore very likely to be more variable than is the alcohol-insoluble fraction. Many known constituents of this soluble fraction, as sugars, the more soluble polysaccharides, organic acids, and the free amino acids, are believed to be completely digestible and the suggestion is made that for practical purposes this fraction be considered completely digestible. The alcohol-soluble matter of most feces amounts to less than 15 percent of the organic matter, and it is unlikely that it is the same soluble matter that was originally present in the forage. The assumption that the soluble matter is completely digestible is a device which enables the calculation of the digestibility of the insoluble matter, discussed in the preceding section.

True Cellulose

Relation of true cellulose to digestibility of dry matter.--This cellulose is considered to be primarily a polymer of glucose and is the chief constituent in all "fibers" studied. It may be considered a "refined" rather than a "crude" fiber. In 46 samples of grass (orchardgrass 18, timothy 8,

bromegrass 14, Kentucky bluegrass 5, and sudangrass 1), the data were:

True cellulose in percent of dry matter (X), 22.1-36.6; Av. 30.7.

Digestion coefficient of dry matter (Y), 50.0-77.5; Av. 67.4.

$r = -0.72^{**}$; $Y = 110.9 - 1.42 X$; S.E. = ± 4.7 ; CV = 7.0.

In 20 samples of alfalfa, the data were:

True cellulose in percent of dry matter (X), 22.1-39.6; Av. 29.4.

Digestion coefficient of dry matter (Y), 47.8-66.8; Av. 60.0.

$r = -0.36$; $Y = 74.5 - 0.49 X$; S.E. = ± 3.8 ; CV = 6.3.

When both grass and alfalfa were considered together, a population of 66:

$r = -0.46^{**}$; $Y = 91.5 - 0.87 X$; S.E. = ± 6.2 ; CV = 9.5.

It is to be noted that there is a negative correlation between the percentage of cellulose and the digestibility of dry matter in grasses, and there is one without significance in alfalfa. These correlation coefficients are not as high as those found for crude fiber, and the errors are slightly larger.

Relation of true cellulose to its own digestibility.--As in the case of crude fiber, if the percentage of cellulose is negatively correlated with dry matter digestibility it could be correlated with its own digestibility, since cellulose constitutes such a large portion of the dry matter. In the same 46 samples of grass, the data were:

Digestion coefficient of true cellulose (Y), 56.1-89.7; Av. 75.4.

$r = -0.79^{**}$; $Y = 135.4 - 1.96 X$; S.E. = ± 5.2 ; CV = 6.9.

In 13 samples of alfalfa the data were:

True cellulose in percent of dry matter (X), 22.1-34.0; Av. 28.7.

Digestion coefficient of true cellulose (Y), 56.4-70.1; Av. 61.9.

$r = -0.06$; $Y = 63.6 - 0.06 X$; S.E. = ± 4.4 ; CV = 7.2.

When the two populations were combined to make a total of 59 samples:

$r = -0.38^{**}$; $Y = 103.3 - 1.02 X$; S.E. = ± 8.8 ; CV = 12.2.

The relation of cellulose to its own digestibility is closer than was that of crude fiber to its own digestibility, but it seems that the relationship is not close enough so that any practical advantage can be made of it.

Natural Cellulose

In the grasses natural cellulose is greater in quantity than true cellulose. It consists of the latter and of certain other polymers containing both hexoses and pentoses. In alfalfa the two celluloses are of the same order of magnitude. The results here are with grasses only, and the percentages have been previously reported (16).

Relation of natural cellulose to digestibility of dry matter.--In 24 samples of grass (orchardgrass 9, bromegrass 6, timothy 5, and Kentucky bluegrass 4), the data were:

Natural cellulose in percent of dry matter (X), 32.7-46.3; Av. 39.1.

Digestion coefficient of dry matter (Y), 57.5-77.2; Av. 69.2.

$r = -0.65^{**}$; $Y = 111.0 - 1.07 X$; S.E. = ± 4.0 ; CV = 5.8.

Relation of natural cellulose to its own digestibility.--In the same 24 samples of grass, the data were:

Digestion coefficient of natural cellulose (Y), 62.4-82.9; Av. 75.4.

$r = -0.60^{**}$; $Y = 119.8 - 1.14 X$; S.E. = ± 4.8 ; CV = 6.3.

The errors are smaller than those obtained with true cellulose. The study on natural cellulose was limited because of the tediousness of the analytical procedure and the general lack of precision of the method.

Total Lignin

Relation of lignin to the digestibility of dry matter.--In 44 samples of grass from two stations (orchardgrass 18, timothy 9, bromegrass 8, Kentucky bluegrass 5, and sudangrass 4), the data were:

Lignin in percent of dry matter (X), 4.7-10.9; Av. 6.9.

Digestion coefficient of dry matter (Y), 50.0-77.5; Av. 67.9.

$r = -0.95^{**}$; $Y = 95.5 - 3.98 X$; S.E. = ± 2.0 ; CV = 3.0.

In 20 samples of alfalfa, the data were:

Lignin in percent of dry matter (X), 8.0-12.6; Av. 9.6.

Digestion coefficient of dry matter (Y), 55.2-66.8; Av. 60.5.

$r = -0.83^{**}$; $Y = 78.3 - 1.86 X$; S.E. = ± 1.8 ; CV = 2.9.

When the two populations were combined to make a total of 64 samples:

$r = -0.93^{**}$; $Y = 90.3 - 3.19 X$; S.E. = ± 2.4 ; CV = 3.7.

The correlation coefficients given by lignin with dry matter digestibility are higher than those given by any other constituent, and the errors of prediction are lower. The regression lines for grass and alfalfa are not alike, particularly as regards slope, but the alfalfa samples available for this study were of relatively high lignin content and of low digestibility so that they fell near the intersection of the two regression lines. When the grasses and alfalfa are considered together, a better agreement is reached between digestibility and lignin than between digestibility and any other constituent considered so far. Figure 5 is a scatter diagram of these results.

In the grasses the few with the highest deviation of the calculated digestibility from that found by the conventional method occurred in grasses that were not first cuttings of the season but aftermath cuttings. In the

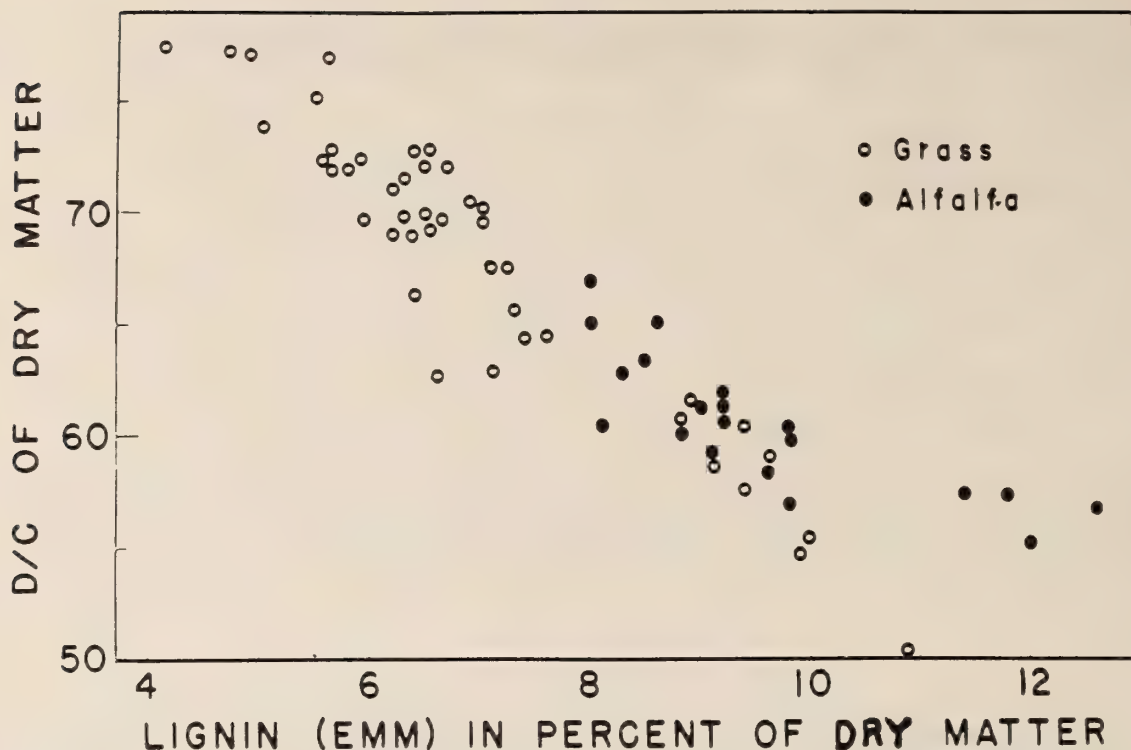


Figure 5.--Relation between total lignin (method of Ellis, Matrone, and Maynard (7)) in percentage of dry matter and the digestion coefficient of dry matter. For the total population, $r = -0.93^{**}$.

search for a common basis of evaluation that would apply to all samples, other relationships were sought. The relation between lignin in percent of organic matter and the digestion coefficient of organic matter gave essentially the same results as those based on dry matter.

Relation of lignin to the digestibility of alcohol-insoluble matter.--As the lignin occurs in the insoluble fraction it should have a greater effect on the digestibility of the alcohol-insoluble matter than on the total dry matter. In 36 grasses, the data were:

Lignin in percent of alcohol-insoluble matter (X), 6.4-14.1; Av. 9.4.

Digestion coefficient of alcohol-insoluble matter (Y), 41.6-77.2;
Av. 63.6.

$r = -0.96^{**}$; $Y = 104.35 - 4.34 X$; S.E. = ± 2.2 ; CV = 3.4.

In 11 samples of alfalfa, the data were:

Lignin in percent of alcohol-insoluble matter (X), 12.6-16.5; Av. 14.0.

Digestion coefficient of alcohol-insoluble matter (Y), 46.6-57.5;
Av. 51.6.

$r = -0.70^{**}$; $Y = 83.4 - 2.27 X$; S.E. = ± 3.2 ; CV = 6.2.

When the two populations were combined to make a total of 47 samples:

$$r = -0.93^{**}; Y = 94.15 - 3.19 X; S.E. = \pm 3.2; CV = 5.3.$$

The results are similar to those with total dry matter.

Relation of lignin to the digestibility of nonprotein alcohol-insoluble matter.--As lignin occurs in the cell wall it should have a greater effect on the nonprotein part of the alcohol-insoluble than on that entire fraction. The relation of lignin (in percent of the insoluble matter) to the nonprotein (carbohydrate) part of the alcohol-insoluble matter was determined on the same 36 samples of grass. The data were:

Digestion coefficient of the nonprotein alcohol-insoluble matter (X),
43.8-77.8; Av. 64.9.

$$r = -0.95^{**}; Y = 103.9 - 4.14 X; S.E. = \pm 2.3; CV = 3.5.$$

In the same 11 samples of alfalfa, the data were:

Digestion coefficient of the nonprotein alcohol-insoluble matter (Y),
42.2-55.1; Av. 48.9.

$$r = -0.81^{**}; Y = 90.7 - 2.99 X; S.E. = \pm 3.1; CV = 6.4.$$

When the two populations were combined to make a total of 47 samples:

$$r = -0.96^{**}; Y = 99.9 - 3.70 X; S.E. = \pm 2.6; CV = 4.2.$$

A scatter diagram of these results is given in Figure 6.

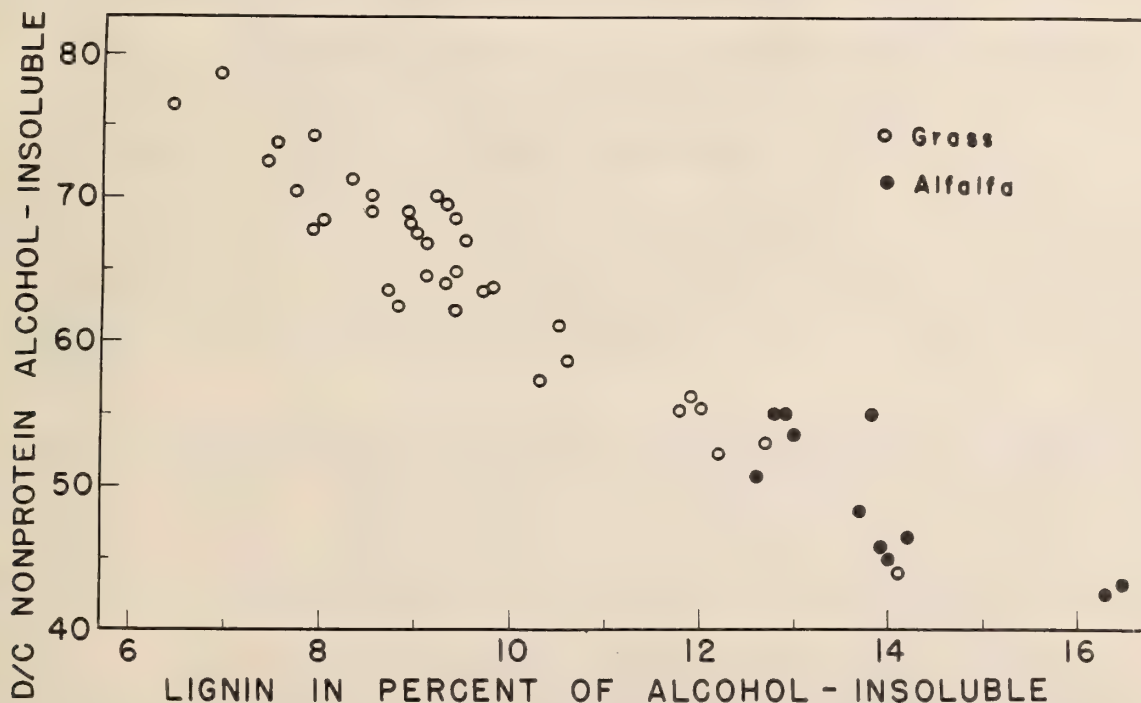


Figure 6.--Relation of total lignin in percent of the alcohol-insoluble matter to the digestion coefficient of the nonprotein alcohol-insoluble matter. For the total population, $r = -0.96^{**}$.

Of the three comparisons (percent lignin to digestibilities of total dry matter, of alcohol-insoluble matter, and of nonprotein alcohol-insoluble matter), the correlations were of about equal value, but the standard errors were smallest in the first case, the relation of lignin to the digestibility of total dry matter. However, it is to be noted that, in the prediction of digestibility of dry matter, the error falls on the entire dry matter, but in the prediction of the digestibility of an insoluble fraction the error falls on that fraction only and not on the soluble fraction. This observation will be made use of in an application to be discussed later. When lignin was compared with the digestion coefficient of nonprotein alcohol-insoluble matter, there was no advantage in expressing the lignin in percent of nonprotein alcohol-insoluble matter instead of in percent of the total alcohol-insoluble matter.

Relation of lignin to the digestibility of crude protein.--If lignin occurs only in the cell wall material, it should have little direct effect on the apparent digestibility of protein except that it may hinder the entrance of bacteria through the cell wall. In the same 36 samples of grasses just mentioned, the data were:

Lignin in percent of dry matter (X), 4.1-10.9; Av. 6.7.

Digestion coefficient of crude protein (Y), 34.6-80.0; Av. 65.4.

$r = -0.86^{**}$; $Y = 107.3 - 6.24 X$; S.E. = ± 5.5 ; CV = 8.4.

In 11 samples of alfalfa, the data were:

Lignin in percent of dry matter (X), 8.0-12.0; Av. 9.6.

Digestion coefficient of crude protein (Y), 68.6-79.1; Av. 74.2.

$r = -0.46$; $Y = 84.2 - 1.04 X$; S.E. = ± 2.7 ; CV = 3.7.

When the two populations were combined to make a total of 47 samples:

$r = -0.32$; $Y = 80.0 - 1.70 X$; S.E. = 9.8; CV = 14.4.

There is a negative correlation between lignin and protein digestibility in grasses and, but to a nonsignificant extent, in alfalfa. The regression equations are so far apart that an acceptable common equation does not exist for predicting digestibility of protein from lignin in grasses, alfalfa, and mixtures.

Relation of lignin to the digestibility of alcohol-insoluble protein.--The effect of lignin on the digestibility of the alcohol-insoluble protein was also calculated. In the same 36 grasses the data were:

Lignin in percent of alcohol-insoluble matter (X), 6.9-12.2; Av. 9.4.

Digestion coefficient of insoluble protein (Y), 3.3-75.7; Av. 53.3.

$r = -0.90^{**}$; $Y = 133.1 - 8.48 X$; S.E. = 7.3; CV = 13.6.

The correlation between these quantities is high but the error of prediction is also high. In the same 11 samples of alfalfa:

$r = -0.07$; $Y = 108.1 - 3.39 X$; S.E. = ± 7.5 ; CV = 12.4.

For the combined population:

$$r = -0.33^* ; Y = 75.4 - 1.94 X ; S.E. = 14.0 ; CV = 25.4.$$

The effect of lignin on the digestibility of insoluble protein is no greater than its effect on crude protein. Although the errors in predicting the digestibility of protein from lignin are high, there is some relationship between them in grasses, but not in alfalfa. The relationship probably results from the fact that the availability of the protein to the digesting bacteria is subject to prior penetration of the cell wall, and this penetration is affected adversely by lignification.

Acid-insoluble Lignin

The acid-insoluble lignin differs from total lignin in that it does not include that part of the lignin that dissolves in the 72 percent sulfuric acid and which is reprecipitated when the acid is diluted with water. The acid-insoluble lignin should be, theoretically, of less quantity than total lignin. The percentages of acid-insoluble lignin are lower than those of total lignin in grasses but they are of the same order in alfalfa.

Relation of acid-insoluble lignin to the digestibility of dry matter.--In 101 grass samples (bromegrass 32, orchardgrass 22, ryegrass 22, Kentucky bluegrass 12, timothy 9, and sudangrass 4), the data were:

Acid-insoluble lignin in percent of dry matter (X), 1.8-7.7; Av. 5.0.

Digestion coefficient of dry matter (Y), 48.1-85.2; Av. 69.3.

$$r = -0.88^{**} ; Y = 96.2 - 5.25 X ; S.E. = \pm 3.6 ; CV = 5.1.$$

In 27 samples of alfalfa, the data were:

Acid-insoluble lignin in percent of dry matter (X), 7.5-12.9; Av. 9.8.

Digestion coefficient of dry matter (Y), 52.6-66.8; Av. 60.5.

$$r = -0.75^{**} ; Y = 78.6 - 1.84 X ; S.E. = \pm 2.3 ; CV = 3.8.$$

In 14 samples of mixed grass and alfalfa, the data were:

Acid-insoluble lignin in percent of dry matter (X), 5.4-9.8; Av. 7.5.

Digestion coefficient of dry matter (Y), 51.0-73.3; Av. 63.4.

$$r = -0.67^{**} ; Y = 85.4 - 2.93 X ; S.E. = \pm 3.9 ; CV = 6.2.$$

When the three populations were combined to make a total of 142 samples:

$$r = -0.77^{**} ; Y = 83.2 - 2.61 X ; S.E. = \pm 4.6 ; CV = 6.9.$$

Other data on acid-insoluble lignin have been published (17). Higher correlation coefficients and lower standard errors were obtained with a smaller population of grasses when all the samples were obtained from the same station. A general equation of

$$Y = 100 - 6 X$$

was proposed in that report for the prediction of digestible dry matter from the percentage of acid-insoluble lignin in grasses (not including reed canary-grass). The difference between the two regression equations, for grass and alfalfa, was so great that it did not seem possible that one equation could be used for both types of forage.

Relation of acid-insoluble lignin to the digestibility of alcohol-insoluble matter.--In 42 samples of grass (orchardgrass 18, bromegrass 8, timothy 7, Kentucky bluegrass 5, and sudangrass 4), the data were:

Acid-insoluble lignin in percent of alcohol-insoluble matter (X),
5.3-9.9; Av. 7.4.

Digestion coefficient of alcohol-insoluble matter (Y), 41.6-77.2;
Av. 63.3.

$r = -0.82^{**}$; $Y = 95.6 - 4.37 X$; S.E. = ± 4.6 ; CV = 7.3.

In 27 samples of alfalfa, the data were:

Acid-insoluble lignin in percent of alcohol-insoluble matter (X),
12.4-16.8; Av. 14.5.

Digestion coefficient of the alcohol-insoluble matter (Y), 41.4-57.5;
Av. 51.1.

$r = -0.21$; $Y = 60.8 - 0.67 X$; S.E. = ± 4.0 ; CV = 7.8.

When the two populations were combined to make 69 samples:

$r = -0.79^{**}$; $Y = 77.65 - 1.88 X$; S.E. = ± 5.4 ; CV = 9.3.

The acid-insoluble lignin is no more closely related to the digestibility of the alcohol-insoluble fraction than it is to the total dry matter. Moreover, the regression equations for the two types of forage are not similar.

Relation of acid-insoluble lignin to the digestibility of the nonprotein alcohol-insoluble matter.--The relationship of lignin to the carbohydrate (nonprotein) part of the alcohol-insoluble matter was also studied. In the same 42 grass samples, the data were:

Digestion coefficient of the nonprotein alcohol-insoluble matter (Y),
43.8-77.8; Av. 64.3.

$r = -0.86^{**}$; $Y = 109.7 - 6.12 X$; S.E. = ± 3.9 ; CV = 6.0.

In the same 27 samples of alfalfa, the data were:

Digestion coefficient of the nonprotein alcohol-insoluble matter (Y),
36.1-56.8; Av. 50.7.

$r = -0.32$; $Y = 68.0 - 1.45 X$; S.E. = ± 5.6 ; CV = 10.9.

When the two populations were combined to give 69 samples:

$r = -0.87^{**}$; $Y = 83.95 - 2.60 X$; S.E. = ± 5.5 ; CV = 9.5.

In the grasses the acid-insoluble lignin was related to the carbohydrate portion of the alcohol-insoluble matter (cell wall material) in much the same way as it was related to the entire insoluble fraction. In alfalfa the relationships were not significant. The relationship of the acid-insoluble lignin to

the protein portion of the alcohol-insoluble matter will be considered in a later section.

Relation of acid-insoluble lignin to the digestibility of crude protein.--In 68 samples of grass (bromegrass 32, orchardgrass 18, timothy 9, Kentucky bluegrass 5, and sudangrass 4), the data were:

Acid-insoluble lignin in percent of dry matter (X), 3.4-7.7; Av. 5.4.

Digestion coefficient of crude protein (Y), 40.9-79.9; Av. 64.7.

$r = -0.55^{**}$; $Y = 94.3 - 5.47 X$; S.E. = ± 7.7 ; CV = 12.0.

In 43 samples of alfalfa, the data were:

Acid-insoluble lignin in percent of dry matter (X), 7.5-12.2; Av. 10.0.

Digestion coefficient of crude protein (Y), 51.9-83.6; Av. 71.3

$r = -0.63^{**}$; $Y = 96.2 - 2.50 X$; S.E. = ± 4.1 ; CV = 5.7.

Acid-insoluble lignin is a better predictor of apparent digestibility of crude protein in alfalfa than it is in grass, as measured by the smaller error. When the two populations were combined, the correlation coefficient was not significant, $r = +0.13$.

Relation of acid-insoluble lignin to the digestibility of the alcohol-insoluble protein.--In 40 samples of grass (orchardgrass 17, bromegrass 8, timothy 6, Kentucky bluegrass 5, and sudangrass 4), the data were:

Acid-insoluble lignin in percent of alcohol-insoluble matter (X), 5.3-9.9; Av. 7.4.

Digestion coefficient of alcohol-insoluble protein (Y), 3.3-75.7; Av. 52.9.

$r = -0.66^{**}$; $Y = 123.8 - 9.58 X$; S.E. = ± 11.7 ; CV = 22.0.

In 27 samples of alfalfa, the data were:

Acid-insoluble lignin in percent of alcohol-insoluble matter (X), 12.4-16.6; Av. 14.5.

Digestion coefficient of alcohol-insoluble protein (Y), 36.8-68.4; Av. 56.4.

$r = -0.48^{*}$; $Y = 96.9 - 2.80 X$; S.E. = ± 6.7 ; CV = 11.9.

When the two populations were combined to make a total of 67 samples, $r = -0.01$.

The relation of acid-insoluble lignin to the apparent digestibility of protein is in general similar to that found with total lignin. Significant correlations in certain cases indicate that lignin does affect to some degree the digestibility of protein, but the errors of prediction are high.

Detergent Lignin

Relation of detergent lignin to the digestibility of dry matter.--For the study of detergent lignin the same samples were used as in that of the detergent fiber. In 36 samples of grass, the data were:

Detergent lignin in percent of dry matter (X), 2.1-6.4; Av. 4.2.

$r = -0.74^{**}$; $Y = 93.8 - 6.12 X$; S.E. = ± 6.0 ; CV = 9.0.

In the 13 samples of alfalfa, the data were:

Detergent lignin in percent of dry matter (X), 4.7-8.4; Av. 7.2.

$r = -0.90^{**}$; $Y = 93.8 - 4.49 X$; S.E. = ± 2.3 ; CV = 3.8.

For 7 mixtures, the data were:

Detergent lignin in percent of dry matter (X), 4.0-7.3; Av. 5.2.

$r = -0.42$; $Y = 67.3 - 0.84 X$; S.E. = ± 2.2 ; CV = 3.5.

When the three populations were combined to make a total of 56 samples:

$r = -0.70^{**}$; $Y = 83.0 - 3.42 X$; S.E. = ± 5.8 ; CV = 8.8.

The two regression equations, for grass and alfalfa, in spite of the agreement as to point of origin, are not alike. The standard errors are high, especially in grasses.

Prediction of Total Digestible Nutrients and Other Quantities

A number of schemes have been published whereby TDN, total digestible organic matter, total energy, or other quantity may be calculated from more than one constituent. Some of these have been investigated. The first ones, which are described below, were developed in this Laboratory and are termed for brevity the "Summation Methods."

Summation method I for calculating total digestible organic matter.--In these methods the organic matter is divided into three fractions, ether extract, alcohol-soluble, and alcohol-insoluble. A number of assumptions are made. The ether extract is assumed to be ash-free and to have a digestion coefficient of 40. Since the ether extract is a relatively small portion of the total organic matter, the errors in these assumptions are negligible. Secondly, the alcohol-soluble portions, including fructosan and starch, are assumed to be completely digestible. Lastly, the digestion coefficients of the alcohol-insoluble fractions vary negatively with the lignin contents and may be predicted from them. In the first of these schemes (Summation I), the digestion coefficient (Y) of the insoluble fraction of a grass is calculated from total lignin (X) by:

$$Y = 104.35 - 4.34 X,$$

an equation offered in an earlier part of this report. The sum of the digestible parts of each fraction is the predicted digestion coefficient of the total organic matter. A sample calculation is given in Table 2.

Table 2.--Sample calculation of Summation I with a timothy having a digestion coefficient of organic matter of 62.4.

Constituent	In % of dry weight	In % of organic matter	In % of alcohol-insoluble matter	Digestion coefficient (x 10 ⁻²)	Digestible nutrient in % of organic matter
<u>Direct</u>					
1. Ash	4.60	---	---	---	---
2. Ether extract	2.94	3.08	---	^a 0.40	1.23
3. Alcohol-insoluble	74.79	78.40	---	^b 0.526	41.24
4. Lignin (EMM)	8.92	---	11.93	---	---
<u>Indirect</u>					
5. Organic matter (100 minus line 1)	95.40	---	---	---	---
6. Alcohol-soluble matter (line 5 minus lines 2 and 3)	17.67	18.52	---	^a 1.00	18.52
				Sum	61.0

^a Assumed values.

^b Calculated from "104.35 - 4.34 x percent lignin."

The digestion coefficients of 36 samples of grass (orchardgrass 16, timothy 8, bromegrass 8, and Kentucky bluegrass 4) were calculated by this scheme. These samples came from the same experiment station, and the digestion data were obtained in nearly each case from four sheep. The digestion coefficients of total organic matter ranged from 51.2 to 80.1, with an average of 70.0. The average recovery of the digestion coefficient of total organic matter by this summation method was 99.8 percent, and the standard error of the difference between the two methods was 1.7; CV = 2.4. This is a very low error, perhaps partly because the work was carried out on samples with a high standard of reliability for the digestion data.

Eleven samples of alfalfa were analyzed by this scheme, with the digestion coefficient of the insoluble fraction (Y) calculated from total lignin (X) by:

$$Y = 83.4 - 2.27 X.$$

The digestion coefficients of total organic matter ranged from 57.7 to 69.1 with an average of 62.2. The average recovery was 100.5 percent and the standard error was 2.5; CV = 4.0.

When the grass and alfalfa were combined into one population of 47, the digestion coefficient of the insoluble fraction (Y) was calculated from total lignin (X) by:

$$Y = 94.15 - 3.19 X.$$

The average recovery of the digestion coefficient of total organic matter by Summation I in a mixed population was practically 100 percent with a standard error of 2.5; CV = 3.7.

The significant result is that the coefficients of variation (CV's) are relatively low and especially that the one for the combined population is low. While the CV for total is higher than the one for grass, it is not higher than the one for alfalfa. Also interesting is that the CV's for the summation are less than when the lignin was used for the prediction of the digestibility of the alcohol-insoluble matter only. The explanation of this could be that the error applies to the alcohol-insoluble fraction alone, which is only part of the forage, and none of the error applies to the alcohol-soluble fraction, if our assumption is admissible that the latter is completely digestible. Another point is that this is one of the few times when the coefficient of variation of the total population does not greatly exceed that for the grass alone or alfalfa alone.

Summation II.--Because the lignin has closer contact to the carbohydrate portion of the alcohol-insoluble fraction than it has to the protein part, another similar scheme was worked out to take advantage of that difference. The second scheme is more elaborate than the first because digestion coefficients of both carbohydrate and protein portions of the alcohol-insoluble are taken into account. In the same 36 samples of grass the digestion coefficients of the two portions were calculated by these equations:

$$\text{Carbohydrate portion, } Y = 103.9 - 4.14 X,$$

$$\text{Protein portion, } Y = 133.1 - 8.48 X,$$

when X is lignin in percent of insoluble matter. The prediction of protein digestibility from lignin was shown earlier to be inaccurate but the error falls on the protein only. The average recovery by Summation II in the grasses was 99.8 percent of the digestion coefficient of total organic matter, and the standard error was 1.9; CV = 2.7.

In the same 11 samples of alfalfa, the digestion coefficients of the insoluble portions were calculated from the lignin, as follows:

$$\text{Carbohydrate portion, } Y = 90.7 - 2.99 X,$$

$$\text{Protein portion, } Y = 108.1 - 3.39 X.$$

The average recovery was 99.2 percent and the standard error was 3.2; CV = 5.1. For the combined populations the following equations were used:

Carbohydrate portion, $Y = 99.9 - 3.70 X$,

Protein portion, $Y = 75.4 - 1.94 X$.

The average recovery was 99.3 percent and the standard error was 2.7; CV = 4.0. No advantage is seen of Summation II over Summation I. More labor is involved and the errors are not smaller.

Summation III.--A third scheme was devised in which a number of assumptions were made. Digestible crude protein was calculated from crude protein by the following equation, according to Reid (14):

$$Y = -3.52 + 0.946 X,$$

where X is the percent of crude protein. The nonprotein portion of the alcohol-soluble was considered completely digestible. The nonprotein portion of the alcohol-insoluble was calculated from the total lignin in a similar manner as before. Summation III is more complex than Summations I and II because it requires two protein determinations. A sample calculation is given in Table 3.

In grass the digestion coefficients of the nonprotein alcohol-insoluble matter were calculated by the equation:

$$Y = 103.9 - 4.14 X,$$

X being total lignin in percent of alcohol-insoluble matter. In 36 samples of grass the average recovery of the digestion coefficient of total organic matter was 100.1 percent. The standard error was 1.7; CV = 2.4.

In 11 samples of alfalfa, the equation to predict the nonprotein insoluble matter was:

$$Y = 90.7 - 2.99 X.$$

The average recovery of digestible organic matter was 100.8 percent and the standard error was 2.1; CV = 3.4.

When both populations were combined, the proper equation was:

$$Y = 99.9 - 3.70 X.$$

The average recovery was 100.3 percent. The standard error was 1.9; CV = 3.1.

These schemes give encouraging results. The errors for the combined population were relatively lower than for each forage by itself. The errors by the more complex scheme, Summation III, were less than by the others. The schemes were also calculated by using acid-insoluble lignin instead of total lignin. With acid-insoluble lignin the results were satisfactory for each class of forage by itself, but larger errors occurred when both types of forage were combined into one population.

Prediction of total digestible nutrients (TDN) from crude protein and crude fiber.--This method of evaluation is being used in some state extension programs (1). Two analyses are made for crude protein (P) and for crude fiber (F). The scheme makes use of three published formulae, those of Reid (14),

Table 3.--Sample calculation of Summation III with a timothy having a digestion coefficient of organic matter of 62.4.

Constituent	In % of dry weight	In % of organic matter	In % of alcohol-insoluble matter	Digestion coefficient (x 10 ⁻²)	Digestible nutrient in % of organic matter
<u>Direct</u>					
1. Ash	4.60	---	---	---	---
2. Ether extract	2.94	3.08	---	^a 0.40	1.23
3. Alcohol-insoluble	74.79	78.40	---	---	---
4. Crude protein	8.24	8.64	---	---	---
5. Alcohol-insoluble protein	6.11	6.40	---	---	---
6. Lignin (EMM)	8.92	---	11.93	---	---
<u>Indirect</u>					
7. Organic matter (100 minus line 1)	95.40	---	---	---	---
8. Nonprotein insoluble matter (line 3 minus line 5)	---	72.00	---	^b 54.51	39.25
9. Digestible protein	^c 4.27	4.48	---	^a 1.00	4.48
10. Alcohol-soluble (100 minus lines 2 and 3)	---	18.51	---	---	---
11. Soluble protein (line 4 minus line 5)	---	2.24	---	---	---
12. Nonprotein soluble matter (line 10 minus line 11)	---	16.27	---	^a 1.00	16.27
Sum					61.2

^a Assumed values.

^b Calculated from "103.9 - 4.14 x percent lignin."

^c Calculated from "-3.52 + 0.946 x percent crude protein" (14).

Axelsson (3), and Swift (18). The formulae were combined arithmetically to give:

$$\text{TDN} = 89.55 + 0.372 \text{ P} - 1.097 \text{ F.}$$

The results obtained by this method for 50 samples in which TDN data were available are given in Table 4. The standard errors are high in comparison with some others, as for example, those with lignin. As a matter of interest it was investigated whether the TDN could be calculated as accurately from crude protein alone or crude fiber alone as from both by the above equation. Regression equations were calculated from these same samples, and the results are summarized in Tables 5 and 6. The standard errors obtained in the calculation of TDN from either crude protein only or from crude fiber only are less than those obtained in the calculation of TDN from both analyses.

Table 4.--Comparison of TDN obtained by conventional method and that calculated from protein and fiber.

Population	Conventional TDN, av.	Calculated TDN, av.	Range of difference	Standard error	Coefficient of variation
42 Grass	64.7	60.6	-14 to + 6	6.9	10.7
7 Alfalfa	61.6	65.6	- 3 to + 11	7.6	12.3
1 Ladino clover	70.5	77.4	---	---	---
50 Total	64.4	61.7	---	6.8	10.6

Table 5.--Calculation of TDN from percent crude fiber (X) on same samples as those in Table 4.

Population	Correlation coefficient	Regression equation	Standard error	Coefficient of variation
42 Grass	-0.61**	TDN = 86.2 - 0.68 X	4.4	6.8
7 Alfalfa	-0.05	TDN = 62.8 - 0.04 X	3.3	5.3
1 Ladino clover	---	---	---	---
50 Total	-0.57**	TDN = 83.9 - 0.63 X	4.6	7.0

Table 6.--Calculation of TDN from percent crude protein (X) on same samples as those in Table 4.

Population	Correlation coefficient	Regression equation	Standard error	Coefficient of variation
42 Grass	+0.66**	TDN = 54.7 + 0.68 X	4.2	6.5
7 Alfalfa	-0.56	TDN = 76.0 - 0.70 X	2.7	4.4
1 Ladino clover	---	---	---	---
50 Total	+0.51**	TDN = 56.3 + 0.52 X	4.8	7.5

Prediction of total digestible organic matter from lignin and nonprotein fat-free organic matter.--A method was proposed by Charlet-Lery et al. (5) for the prediction of the digestion coefficient of organic matter from the ratio of lignin to MTD (matières ternaires delipidées), the MTD being the percentage of organic matter less the percentages of crude protein and ether extract. The MTD is essentially the sum of the crude fiber and the nitrogen-free extract. As Charlet-Lery et al. used a different lignin method, another equation was prepared in this work, based on the total lignin method of Ellis et al. (7). In 44 samples of grass (orchardgrass 18, bromegrass 8, timothy 9, Kentucky bluegrass 5, and sudangrass 4), the data were:

Lignin/MTD ratio (X), 0.0645-0.1260; Av. 0.0905.

Digestion coefficient of organic matter (Y), 51.2-80.1; Av. 69.3

$r = -0.92^{**}$; $Y = 107.2 - 418.4 X$; S.E. = ± 2.7 ; CV = 3.9.

In 11 samples of alfalfa, the data were:

Lignin/MTD ratio (X), 0.1172-0.1752; Av. 0.1390.

Digestion coefficient of organic matter (Y), 55.9-69.1; Av. 62.0.

$r = -0.77^{**}$; $Y = 87.4 - 182.9 X$; S.E. = ± 2.8 ; CV = 4.5.

When the two populations are combined to a total of 55 samples:

$r = -0.82^{**}$; $Y = 91.3 - 233.5 X$; S.E. = ± 4.1 ; CV = 6.1.

This method is essentially one for lignin expressed in percentage of total carbohydrate, the organic matter on which the percentage is based being free from protein and ether extract.

A similar study was also carried out by using the percentage of acid-insoluble lignin. In the same 44 samples of grass, the data were:

Acid-insoluble lignin/MTD ratio (X), 0.0481-0.0926; Av. 0.0700.

Digestion coefficient of organic matter (Y), 51.2-80.1; Av. 69.3.

$$r = -0.77^{**}; Y = 102.2 - 468.4 X; S.E. = \pm 4.9; CV = 7.2.$$

In 11 samples of alfalfa, the data were:

Acid-insoluble lignin/MTD (X), 0.1244-0.1768; Av. 0.1440.

Digestion coefficient of organic matter (Y), 55.9-69.1; Av. 62.0.

$$r = -0.77^{**}; Y = 136.8 - 519.6 X; S.E. = \pm 6.2; CV = 10.0.$$

When the two populations were combined to make a total of 55 samples:

$$r = -0.62^{**}; Y = 79.3 - 134.4 X; S.E. = \pm 5.6; CV = 8.2.$$

The prediction of digestibility of organic matter by the lignin/MTD ratio was slightly less accurate, measured by the standard error, than the prediction of digestibility of dry matter from lignin, as mentioned previously. There was no advantage in computing the ratio. The correlations were less and the errors were greater when acid-insoluble lignin was used in the computation of the ratio than when the total lignin percentages were used.

Prediction of digestible energy from lignin and protein.--Sosulki and Patterson (15) found a relationship between lignin (L) and crude protein (P) and digestible energy (DE). As two equations representing different years were similar to one another, an average one is used here:

$$DE = 77.3 - 4 L + 1.37 P$$

These authors used the lignin method of Ellis et al. (7). Lignin percentages by this method and digestible energy data were available on 39 forage samples, of which 37 were grasses, 1 was alfalfa, and 1 was ladino clover. The digestible energy by the conventional method ranged from 47.3 to 76.0 units, with an average of 65.5. When DE was calculated by the above equation, it ranged from 42.9 to 92.7, with an average of 69.9, which was 4.4 units too high, and the standard error of the difference was 8.4; CV = 12.8. When only those samples that were first cuttings of grasses were considered (26 samples) the calculated DE differed by being only 1.3 units too high on the average, but the error was still high, 6.7; CV = 10.2. The greatest errors were noted in samples which were considerably far from the average in composition; high-protein samples were overestimated by the calculated method and low-protein samples were underestimated. Much greater divergence was noted in aftermath than in first cuttings.

DISCUSSION

What are acceptable methods of evaluating forages by chemical means, and which of the various methods discussed give results closest to true digestion values? If methods are to be compared some uniform system is needed. The different methods predict quantities of different sizes, such as digestibility of total dry matter and percent digestible protein so that the standard errors are not in comparable units. The procedure chosen is to compare coefficients of variation (CV), which, in each case, is the standard error divided by the average value of the predictant. Another basis of evaluation is to compare correlation coefficients. The coefficient of variation is considered to be

superior to the correlation coefficient for the purpose of these studies but because this opinion is not held unanimously, both coefficients are presented.

In Table 7 the coefficients of correlation and variation found in the prediction of digestibility by the protein content are brought together. The percentage of crude protein has been used to predict three entities -- the digestion coefficient of dry matter, the digestion coefficient of crude protein, and the percent of digestible crude protein. It predicts the percent of digestible protein more accurately than it does the other two. Its prediction of total digestible dry matter is subject to considerable error, but the most significant point is that regression equations for grass and for alfalfa are far apart and a single equation is not suitable for mixtures. This results in a higher CV in the "total" category than when one type of forage is considered by itself.

Table 7.--Coefficients of correlation (r) and errors of prediction (CV) by crude protein in percent of dry matter.

Population	Predictant	r	CV
101 Grass	Digestion coefficient of dry matter	+0.61**	8.7
54 Alfalfa	-----do-----	+0.40**	6.3
10 Mixtures	-----do-----	+0.57	7.9
165 Total	-----do-----	+0.24**	10.7
76 Grass	Digestion coefficient of crude protein	+0.85**	7.9
50 Alfalfa	-----do-----	+0.61**	4.6
10 Mixtures	-----do-----	+0.25	6.5
136 Total	-----do-----	+0.86**	6.7
76 Grass	Digestible crude protein in percent of dry matter	+0.99**	4.6
50 Alfalfa	-----do-----	+0.95**	4.4
10 Mixtures	-----do-----	+0.97**	7.1
136 Total	-----do-----	+0.99**	5.0

Crude fiber has been widely used as a criterion of digestibility but has also been severely criticized (8, 11). It was hoped that some modification of it would be found more useful for the evaluation of forages. The different fibers discussed here are the residues of different treatments. Some of the treatments are more drastic than others, requiring different chemical reagents of different solvent powers, at longer duration or higher temperatures. All the methods are empirical, which means, in practice, that the amounts of

substances removed depend on the conditions of treatment, which must be followed exactly, and that the residues (or fibers) have no definite or constant composition. In Figure 7 some of the fibrous residues are illustrated quantitatively. Four forages, two grasses and two alfalfas, having different digestion coefficients, were analyzed for seven different fibers. They are arranged from left to right in decreasing order of the abundance of the fibers. Each vertical legend indicates in a general way what substances have been removed in the production of the fiber as compared with the fiber to the left of it. For example, the treatment which left behind the alcohol-insoluble matter removed all readily soluble substances as lipids, sugars, and other simple organic compounds. The ammonium oxalate fiber has lost, in addition to the above, pectins and small quantities of other hemicellulosic substances. The acetic acid fiber lacks the same substances as does the preceding fiber and in addition a large part of the protein (by the action of the pepsin) and a fair share of the hemicellulose (by the action of the acetic acid). The normal-acid fiber contains still less hemicellulose because of the greater solvent action of the sulfuric acid as compared with the acetic acid, but the normal-acid fiber contains more protein as it was not subjected to the action of pepsin. The detergent fiber has lost about as much protein as did the pepsin-treated acetic acid fiber and as much hemicellulose as did the normal-acid fiber so that it ended up as a smaller quantity than the normal-acid fiber. Cellulose differs from all the preceding fibers in being more nearly a single substance. It contains only minor quantities of hemicelluloses and lignin and practically no protein. Crude fiber is present in quantities close to those of cellulose, though these two are not of the same composition -- the crude fiber containing some lignin and having lost some cellulose. At the extreme right of the figure are plotted the percentages of nondigestible organic matter. The different fibers were intended to be correlated with the nondigestible matter, but none of the fibers resemble graphically the nondigestible organic matter. In no case does the same kind of fiber in the four forages fall into the same order as the percentage of nondigestible matter. In quantity, however, cellulose and crude fiber come closest to the nondigestible matter.

In Table 8 are brought together the coefficients of correlation and variation found in the prediction of digestion coefficients from fibrous constituents. In the grasses no CV's have been found with any of the fibers that are smaller than those with crude fiber, though some high correlation coefficients are noted. With alfalfa, some methods have given smaller CV's than has crude fiber, but the number of samples considered in those cases was relatively small. Wherever the CV for the "total" category was greater than for grass alone or alfalfa alone it seems that a single equation should not be used for both grass and alfalfa.

The percentages of many of these fibers are correlated with their own digestion coefficients. If this relationship were close it could be very useful in predicting the digestibility of the whole forage. If the crude fiber could predict its own digestibility with fair accuracy, the digestibility of a large part of the total dry matter could be predicted, as crude fiber accounts for about a third of the total organic matter. But the prediction of digestion coefficients of a constituent from its own percentage is not successful, as indicated by the high CV's in the "total" categories.

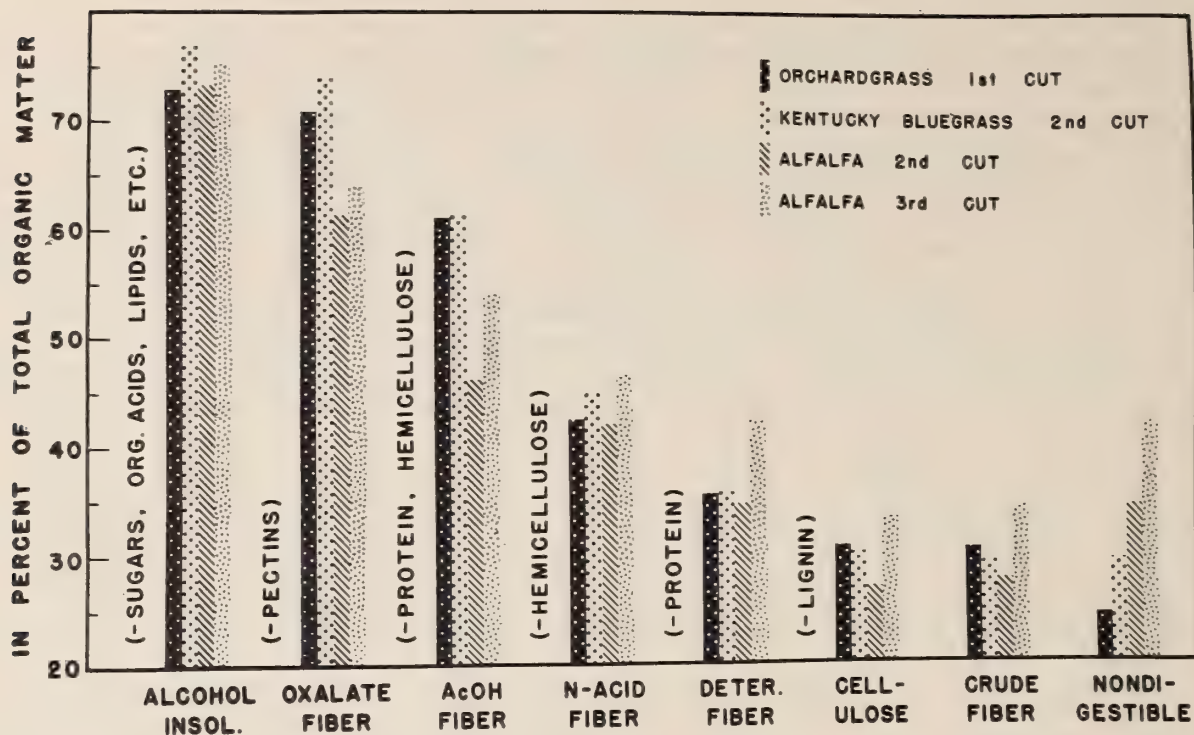


Figure 7.--Bar graphs illustrating seven "fibers," viz., alcohol-insoluble matter, ammonium oxalate fiber, acetic acid fiber, normal-acid fiber, detergent fiber, cellulose, and crude fiber, in four forages. The height of a bar gives the percent of each fiber in total organic matter. The horizontal legends name the fibers, which are arranged in decreasing percentage from left to right. The graph on the extreme right gives nondigestible organic matter, also in percent of total organic matter. Each vertical legend between the bar graphs indicates in a general way the constituents that have been removed in some quantity in the production of the fiber to its right as compared with the one to its left. The percentages of the same kind of fiber in four forages may be compared with the percentages of nondigestible matter. Further explanation is given in the text, p. 43.

Some authors (6) have proposed that cellulose replace crude fiber as the criterion of digestibility in forages. These results show no advantage of cellulose over crude fiber for that purpose, but on the other hand they do not show any serious disadvantage. Considering the greater ease and rapidity of the analytical procedure for cellulose as compared with crude fiber, there is no reason why cellulose (true cellulose) could not replace crude fiber, but for this reason only.

In Table 9 are brought together coefficients of correlation and variation found in the prediction of various digestion coefficients from some form of lignin. The CV's are in general lower than those in Tables 7 and 8. The total lignin method, with the procedure of Ellis et al. (7), will predict the digestion coefficients of dry matter in grass and also in alfalfa with a

Table 8.--Coefficients of correlation (r) and errors of prediction (CV) by methods that determine fiber in various forms.

Population	Predictant	r	CV
<u>Predictor: Crude fiber in percent of dry matter</u>			
65 Grass	Digestion coefficient of dry matter	-0.65**	6.0
20 Alfalfa	-----do-----	-0.54*	5.7
13 Mixtures	-----do-----	+0.49	4.5
98 Total	-----do-----	-0.49**	7.3
38 Grass	Digestion coefficient of crude fiber	-0.61**	7.8
16 Alfalfa	-----do-----	+0.19	15.1
<u>Predictor: Normal-acid fiber in percent of dry matter</u>			
25 Grass	Digestion coefficient of dry matter	-0.79**	8.5
9 Alfalfa	-----do-----	-0.91**	2.7
34 Total	-----do-----	-0.71**	9.8
<u>Predictor: Acetic acid fiber in percent of dry matter</u>			
20 Grass	Digestion coefficient of dry matter	-0.86**	6.6
9 Alfalfa	-----do-----	-0.95**	3.0
29 Total	-----do-----	-0.70**	12.5
<u>Predictor: Detergent fiber in percent of dry matter</u>			
36 Grass	Digestion coefficient of dry matter	-0.78**	8.1
13 Alfalfa	-----do-----	-0.83**	4.9
7 Mixtures	-----do-----	+0.87**	1.9
56 Total	-----do-----	-0.76**	8.1
<u>Predictor: Ammonium oxalate fiber in percent of dry matter</u>			
21 Grass	Digestion coefficient of dry matter	-0.83**	7.3
9 Alfalfa	-----do-----	-0.62	3.4
33 Total	-----do-----	-0.26	12.2

Table 8 (continued)

Population	Predictant	r	CV
<u>Predictor: Protein-free ammonium oxalate fiber</u> <u>in percent of dry matter</u>			
21 Grass	Digestion coefficient of dry matter	-0.86**	6.5
9 Alfalfa	-----do-----	-0.70*	3.1
33 Total	-----do-----	-0.41*	10.9
<u>Predictor: Alcohol-insoluble matter in percent</u> <u>of organic matter</u>			
67 Grass	Digestion coefficient of organic matter	-0.63**	7.2
25 Alfalfa	-----do-----	-0.67**	5.0
92 Total	-----do-----	-0.39**	9.0
43 Grass	Digestion coefficient of alcohol-insoluble matter	-0.58**	10.0
18 Alfalfa	-----do-----	-0.34	6.8
61 Total	-----do-----	-0.32*	14.0
<u>Predictor: Nonprotein alcohol-insoluble matter</u> <u>in percent of organic matter</u>			
67 Grass	Digestion coefficient of organic matter	-0.76**	6.1
25 Alfalfa	-----do-----	-0.73**	4.6
92 Total	-----do-----	-0.44**	8.9
44 Grass	Digestion coefficient of nonprotein alcohol-insoluble matter	-0.76**	7.8
15 Alfalfa	-----do-----	-0.44	12.8
59 Total	-----do-----	-0.27*	17.0
<u>Predictor: True cellulose</u>			
46 Grass	Digestion coefficient of dry matter	-0.72**	7.0
20 Alfalfa	-----do-----	-0.36	6.3
66 Total	-----do-----	-0.46**	9.5
46 Grass	Digestion coefficient of true cellulose	-0.79**	6.9
13 Alfalfa	-----do-----	-0.06	7.2
59 Total	-----do-----	-0.38**	12.2

Table 8 (continued)

Population	Predictant	r	CV
<u>Predictor: Natural cellulose</u>			
24 Grass	Digestion coefficient of dry matter	-0.65**	5.8
24 Grass	Digestion coefficient of natural cellulose	-0.60**	6.3

standard error of about 2 units and a CV of about 3 percent. These errors are probably in the range of accuracy of the digestion trials on which the data are based. Moreover, the samples on which this study was based are those that the author feels are the most reliable as far as digestion trials were concerned. They are nearly all from the same station, and three or four animals were used in each. A point in favor of this lignin method is that the CV's in the "total" category are not much higher than those in the individual categories. It may be seen in Figure 5 that most of the alfalfa samples were in general higher in lignin and lower in digestibility than most of the grass samples and had a narrower range in variation than the grasses. The majority of the alfalfa samples fell near the intersection of the two regression lines.

The chief criticisms of the total lignin method are that it takes considerable time to carry out and its precision is not high. These drawbacks have discouraged its use, and more rapid methods are being sought, two of which were studied in this work.

The method for acid-insoluble lignin requires less labor than that for total lignin. It was carried out on more samples than was total lignin, which were from more sources, and had digestion data that were not replicated to the same degree. This may explain in part the higher CV's obtained. Lower errors than these reported here were obtained in a previous study (17) with a more limited number of samples. In that report, 60 grass samples, ranging from 3.4 to 7.7 percent in acid-insoluble lignin and 50 to 78 in digestion coefficients of dry matter, gave a correlation coefficient of -0.90** between those two quantities, with a standard error of 2.8 and a CV of 4.3. This suggests that acid-insoluble lignin may be used in predicting digestibility of dry matter in a number of species of grass (reed canarygrass was an exception). The effect of acid-insoluble lignin on digestibility in a more limited number of alfalfa samples shows a lower regression factor and a moderate CV. On a scatter diagram for alfalfa, the points are distributed along a line that is nearly horizontal, so that extreme accuracy is required for the prediction of digestibility. However, the two regression equations, for grass and alfalfa, do not resemble each other and a common equation is not feasible. This author has received opinions from others who use the acid-insoluble lignin method that its precision is not high.

Table 9.--Coefficients of correlation (r) and errors of prediction (CV) by lignin in various forms.

Population	Predictant	r	CV
<u>Predictor: Total lignin in percent of dry matter</u>			
44 Grass	Digestion coefficient of dry matter	-0.95**	3.0
20 Alfalfa	-----do-----	-0.83**	2.9
64 Total	-----do-----	-0.93**	3.7
36 Grass	Digestion coefficient of crude protein	-0.86**	8.4
11 Alfalfa	-----do-----	-0.46	3.7
47 Total	-----do-----	-0.32	14.4
36 Grass	Digestion coefficient of insoluble protein	-0.90**	13.6
11 Alfalfa	-----do-----	-0.07	12.4
47 Total	-----do-----	-0.33*	25.4
<u>Predictor: Total lignin in percent of alcohol-insoluble matter</u>			
36 Grass	Digestion coefficient of alcohol-insoluble matter	-0.96**	3.4
11 Alfalfa	-----do-----	-0.70**	6.2
47 Total	-----do-----	-0.93**	5.3
36 Grass	Digestion coefficient of nonprotein alcohol-insoluble matter	-0.95**	3.5
11 Alfalfa	-----do-----	-0.81**	6.4
47 Total	-----do-----	-0.96**	4.2
<u>Predictor: Acid-insoluble lignin in percent of dry matter</u>			
101 Grass	Digestion coefficient of dry matter	-0.88**	5.1
27 Alfalfa	-----do-----	-0.75**	3.8
14 Mixtures	-----do-----	-0.67**	6.2
142 Total	-----do-----	-0.77**	6.9
68 Grass	Digestion coefficient of crude protein	-0.55**	12.0
43 Alfalfa	-----do-----	-0.63**	5.7

Table 9 (continued)

Population	Predictant	r	CV
<u>Predictor: Acid-insoluble lignin in percent of alcohol-insoluble matter</u>			
42 Grass	Digestion coefficient of alcohol-insoluble matter	-0.82**	7.3
27 Alfalfa	-----do-----	-0.21	7.8
69 Total	-----do-----	-0.79**	9.3
42 Grass	Digestion coefficient of nonprotein alcohol-insoluble matter	-0.86**	6.0
27 Alfalfa	-----do-----	-0.32	10.9
69 Total	-----do-----	-0.87**	9.5
40 Grass	Digestion coefficient of insoluble protein	-0.66**	22.0
27 Alfalfa	-----do-----	-0.48*	11.9
<u>Predictor: Detergent lignin in percent of dry matter</u>			
36 Grass	Digestion coefficient of dry matter	-0.74**	9.0
13 Alfalfa	-----do-----	-0.90**	3.8
7 Mixtures	-----do-----	-0.42	3.5
56 Total	-----do-----	-0.70**	8.8

The detergent lignin method is the most rapid of the three methods. One boiling with the combination of the acid detergent and sulfuric acid removes the greater part of the ether-soluble material, protein, and hemi-cellulose in one step. The lower percentages found of detergent lignin than of acid-insoluble lignin suggest that some of the latter may be dissolved by the detergent. The study of the detergent method has been more limited than those of the other lignins, and should be expanded.

It was noted that two lignin procedures, for total lignin and acid-insoluble lignin, gave different values in grass but similar values in alfalfa. When a single equation is desired for computing digestible matter in both grasses and alfalfa the one based on total lignin serves best. However, as the procedure for insoluble lignin is more rapid, it is suggested that in a program of evaluating forages of different types, some grasses and some alfalfa, the total lignin procedure could be used for the grasses and the

acid-insoluble procedure for the alfalfa. When this was tested against 44 grass and 27 alfalfa samples, the following results were obtained:

$$r = -0.91^{**}; Y = 88.5 - 2.92 X; S.E. = \pm 2.7; CV = 4.2.$$

Such a scheme would have merit in cutting the time of analysis in alfalfa samples only. Which procedure to follow on mixed samples would depend on whether the grass or alfalfa predominated in a particular sample.

The CV's obtained in the study of miscellaneous methods are brought together in Table 10. Summation I rests upon the separation of the forage sample into three fractions by the solvents ether and alcohol and on the lignin content. Summations II and III involve more labor than Summation I because of the two protein determinations; but one of them, that of crude protein, would probably be made by the analyst in any case. In Summation III the digestible protein is calculated by an equation borrowed from another source (14) and not derived from the samples reported here.

All three summation methods give excellent predictions of total organic matter digestibility. Because the errors of the methods are not much lower than those obtained when the prediction is based on lignin only, it is questionable whether the extra labor required by the solvent extractions is worth the effort. This last statement applies only to forage plants in which the soluble fraction falls into the normal pattern. If the soluble fraction is abnormal because of some external condition or method of preservation so that the ratio of lignin to total organic matter is altered unduly but its ratio to insoluble matter is unchanged, a summation method might be worth the effort.

The prediction of TDN from both crude protein and crude fiber gives high CV's and should not be further considered. The prediction of the digestion coefficient of organic matter by the lignin/MTD ratio depends essentially on the lignin determination, and the results resemble other results that depend on lignin. No advantage is seen in the ratio method of expression. Also no advantage is seen in the prediction of digestible energy from lignin and protein.

It is obvious from the results that the chief difficulty in predicting digestibility of a forage from its chemical composition lies in the difference between a grass and a legume. In these studies alfalfa is practically the only legume that has entered into the botanical composition of the forages. It is obvious that when the composition of a grass and a legume is compared all variables other than species which affect composition cannot be eliminated. The only recourse is to compare averages of large numbers of samples. As the grass samples greatly outnumber the alfalfa samples the data reported here may not be true averages. In any case it appears (from Table 1) that grasses (without designating species) are higher than alfalfa in percentages of ether extract and certain of the fibers, as acetic acid fiber and ammonium oxalate fiber, and in the nonprotein portions of the ammonium oxalate fiber and the alcohol-insoluble matter. Grass also exceeds alfalfa in the digestion coefficients of total dry matter, ether extract, and all the fibers studied, which include crude fiber, true cellulose, alcohol-insoluble matter, and non-protein alcohol-insoluble matter. However, alfalfa is higher than grass in

Table 10.--Coefficients of variation in the prediction of digestibility by more than one analysis.

Population	Predictant	CV
36 Grass	Digestion coefficient of organic matter by Summation I	2.4
11 Alfalfa	-----do-----	4.0
47 Total	-----do-----	3.7
36 Grass	Digestion coefficient of organic matter by Summation II	2.7
11 Alfalfa	-----do-----	5.1
47 Total	-----do-----	4.0
36 Grass	Digestion coefficient of organic matter by Summation III	2.4
11 Alfalfa	-----do-----	3.4
47 Total	-----do-----	3.1
42 Grass	TDN by crude protein and crude fiber-----	10.7
7 Alfalfa	-----do-----	12.2
50 Total	-----do-----	10.6
44 Grass	Digestion coefficient of organic matter by lignin/MTD	3.9
11 Alfalfa	-----do-----	4.5
55 Total	-----do-----	6.1
44 Grass	Digestion coefficient of organic matter by acid-insoluble lignin/MTD	7.2
11 Alfalfa	-----do-----	10.0
55 Total	-----do-----	8.2
39 Total	Digestible energy by lignin and protein-----	12.8
26 First-cut grass	-----do-----	10.2

both crude protein and the digestibility of crude protein. Slightly but perhaps not significantly greater portions of the total crude protein were found in the alcohol-soluble fraction of alfalfa than of grass. In short, alfalfa, as compared to grass, is higher in protein and, in these samples, about equal in fiber. Its protein has a higher digestibility and its fiber a lower digestibility. This gives alfalfa a superiority in terms of protein but not in total energy-producing substances. Lignin is higher in alfalfas than in grass and, as lignin inhibits digestibility, the alfalfas with a higher

lignin content than grass have, in general, a lower digestibility than grass, but the last is not always the case. This is our greatest difficulty. The relation of composition to digestibility is not the same in both kinds of forage, and a common criterion has not been found.

There are undoubtedly differences in chemical composition among species of grass. In the work reported here the numbers of samples of each species are perhaps insufficient for adequate comparisons. The point at issue is not the differences among species in composition but the relation of composition to digestibility and differences in this respect between species are not readily recognized. If it should be true that the grasses common to the Northeastern United States do not differ in any marked degree from one another in relation of chemical composition to nutritive value, it would be fortunate and make possible their evaluation by a common analytical procedure. In this report no distinction was made among grass species. All were grouped together, but the number of each species was recorded for each test.

The most important factor in the quality of forage is the stage at which the forage is harvested. There is no record in many cases as to the date of cutting and the stage of growth at that time. The majority of samples, especially the grasses, represent the first cutting of the season. The composition of the first cutting and digestibility are closely related. As grass matures its composition changes in a fairly regular manner, and the changes are reflected in the composition of the harvested forage. It is not surprising that correlations, either positive or negative, are common among constituents. Digestion coefficients also change in a regular manner as grass matures, and correlations between composition and digestibility result as a matter of course. If these relationships were very great, there would be no problem in ascertaining the quality of a forage from its date of harvest and its composition. But the relationships are modified by many variables, such as the weather, soil fertility, genetic variability among plants, especially those that affect rate of maturation, and the conditions of preservation. It is the individual sample on hand that needs to be evaluated and not the ideal one.

There were an insufficient number of samples on hand to settle an interesting point -- whether second or later cuttings differ from first cuttings in quality when the chemical composition of the two are similar. In a few cases the coefficients of variation in some comparisons were greatest in late-season cuttings, but this was not true in many other cases. The time elapsed between cuttings is of importance in determining the quality of a forage as well as its composition. The relation between the two, quality and composition, seems to be the same in these aftermath cuttings as in first cuttings, but it is possible that they are not the same.

Many questions remain unanswered in this study, which has been limited as to the techniques used, the kinds of forage, and the information available on each sample. Additional work is needed to answer some of the questions: Is the relationship between chemical composition and digestibility the same in all species of grass, in strains within a species, in aftermath as well as first cuttings, in summer and fall as in spring, in annuals as well as in

perennials, and under different cultural practices? Should the same analytical procedures be applied to silages as to hays?

New analytical methods and more rapid procedures with particular emphasis on precision need to be developed.

The reliability of the digestion data of many of the samples included in this study may be open to question. The author has no reason for rating some data above others except on the basis of number of animals used. Even after well-conducted digestion trials, some error is present in the digestion coefficients attached to a particular sample and the question is whether too much is expected in the chemical evaluation of forages. Should, for example, a method that gives an error measured by a coefficient of variation of 5 or 6 be considered satisfactory or should a method that gives one of 2 or 3 be sought? Should some other goal be established rather than the estimation of digestibility? Should not studies on chemical composition be directed toward the prediction of voluntary intake, body-weight gains, or milk production rather than digestion coefficients?

SUMMARY

1. This report gives the results of studies on the relation of chemical composition of forages to their nutritive value. More specifically, the report deals with the prediction of digestion coefficients from the quantity of certain chemical constituents or fractions.

2. The methods used in the chemical analyses are described. The analyses were restricted to those constituents or fractions believed to be of importance in the digestion of forages by ruminants.

3. The materials available for this study were dried samples of forages donated by agricultural experiment stations, located chiefly in the North-eastern United States but also in other states and in the United Kingdom. The samples were of grasses and of alfalfa, and their digestion coefficients of dry matter or organic matter had been determined in the conventional manner with either cattle or sheep.

4. A summary table (see Table 1) lists the constituents determined, their average content in grasses and in alfalfa, and some of their digestion coefficients.

5. The percentage of crude protein was positively correlated with the digestion coefficients of dry matter and of crude protein itself and with the percentage of digestible protein (see Table 7). The percentage of crude protein will predict the digestion coefficient of dry matter with an error, expressed as the coefficient of variation (CV) between the predicted and the known digestion coefficients, of about 8.

6. The percentage of crude protein will predict the digestion coefficient of crude protein with a CV of about 8 in grasses and of less than 5 in alfalfa.

7. The percentage of crude protein was very highly correlated with the percentage of digestible protein, $r = +0.99$ for all forages. The percentage of digestible protein (Y) may be predicted from the percentage of crude protein (X) by

$$Y = -2.79 + 0.88 X$$

for all forages, with a CV of 5 or less. Wherever crude protein was used as a predictor, it served more accurately in alfalfa than in grass, as indicated by a smaller CV.

8. The percentage of crude fiber was negatively correlated with the digestion coefficient of dry matter (see Table 8). The digestion coefficient of dry matter may be predicted from the percentage of crude fiber with a CV of about 6. The percentage of crude fiber was negatively correlated with its own digestibility, which may be predicted with a CV in excess of 8 in grasses. The error of prediction was greater in alfalfa.

9. Other "fibers" were studied for the purpose of finding a fiber that might be more accurate than crude fiber in the prediction of digestibility. In the grasses, normal-acid fiber, acetic acid fiber, detergent fiber, ammonium oxalate fiber, and nonprotein ammonium oxalate fiber (cell wall material), all being named in accordance with the procedure by which they were prepared, when used to predict the digestion coefficient of dry matter, gave CV's that were higher than those given by crude fiber. In all cases, higher CV's were found for mixed populations than when grasses only or alfalfa only were considered. This last condition illustrated that a single prediction equation was unsatisfactory for both grasses and alfalfa.

10. The percentage of cellulose, determined by the method of Crampton and Maynard, was negatively correlated with the digestion coefficient of dry matter and may be used for its prediction with CV's of about the same order as those obtained with crude fiber. The percentage of cellulose is slightly more closely related to its own digestibility than is crude fiber to its own digestibility. Considering the similarity of the two substances in quantity and in relation to their digestion coefficients, it appears possible that this form of cellulose (which is determined with relative ease) could be used in place of the more time-consuming crude fiber as a criterion of quality. Cellulose is not superior to crude fiber in any other respect than in its ease of determination.

11. A new method is proposed for the separation of a forage into three fractions based on their solubility in ether and 80 percent alcohol, viz., ether-soluble, alcohol-soluble, and alcohol-insoluble. A mild acid hydrolysis (1 percent oxalic acid) previous to the separation by alcohol allows the addition to the alcohol-soluble fraction of some substances, as fructosan and starch, of high digestibility. The alcohol-soluble fraction is positively correlated with the digestibility of the total dry matter, and the alcohol-insoluble fraction is negatively correlated with it. The assumption that the

alcohol-soluble material is completely digestible is a device that allows the calculation of the digestion coefficient of the alcohol-insoluble fraction.

12. The percentage of total lignin, determined by the method of Ellis, Matrone, and Maynard, was negatively correlated with the digestion coefficient of dry matter, the coefficients being -0.95 in grasses and -0.83 in alfalfa (see Table 9). These are higher correlation coefficients than were obtained between any other constituent and the digestibility of dry matter. The percentage of total lignin will predict the digestion coefficient of dry matter with a CV of 3.0 in grasses, 2.9 in alfalfa, and 3.7 in the combined populations. These are lower CV's than were obtained in the prediction of dry matter digestibility from any other constituent. Also, the percentage of total lignin will predict the digestion coefficient of various fibers and insoluble fractions with relatively low CV's. The percentage of total lignin was also negatively correlated with the digestion coefficients of crude protein and of insoluble protein and it will predict their digestion coefficients but not with the accuracy with which it will predict the digestibility of substances consisting mainly of carbohydrate.

13. The percentages of acid-insoluble lignin were less than those of total lignin in the grasses, but the two were of about the same order in alfalfa. Acid-insoluble lignin, in a larger and more varied population, gave lower correlation coefficients with digestibility than did total lignin and higher CV's in the prediction of digestion coefficients, but the acid-insoluble lignin was superior to all forms of fiber in these respects. In a relatively small and selected population, acid-insoluble lignin is only slightly inferior to total lignin in predicting the digestion coefficients of dry matter in grasses. But it will not serve nearly so well when a single prediction equation is applied to both grasses and alfalfa. The statements in regard to acid-insoluble lignin may apply in general to detergent lignin as well.

14. Schemes named Summation Methods are proposed for the calculation of total digestible organic matter. All depend on the assumption that ether extracts have a digestion coefficient of 40, that the alcohol-soluble matter is completely digestible, and that the digestibility of alcohol-insoluble matter may be predicted from its lignin content. The sum of the digestible portions is the total digestible organic matter. In the first of these schemes, Summation I, applied to 47 samples containing grasses and alfalfa, the sum of the digestible portions agreed with the total digestible organic matter obtained in the conventional manner, with a coefficient of variation of 3.7. In another more complex scheme, Summation II, the digestibilities of both the protein and nonprotein portions of the alcohol-insoluble matter have been calculated separately from the lignin content. The sum of the digestible portions agreed with total digestible organic matter with a coefficient of variation of 4.0. In a third scheme, Summation III, four digestible fractions were determined. The digestible protein is calculated from the total crude protein and the soluble and insoluble carbohydrates were considered separately. The sum of the digestible portions agreed with the total digestible organic matter with a coefficient of variation of 2.8. Examples are given for the calculations involved in these schemes.

15. A number of schemes proposed by others for the calculation of digestible organic matter, digestible energy, and TDN from two or three constituents were investigated with a limited number of samples. None of them seemed more suitable to the prediction of digestibility than those in which a single constituent was used.

16. Some problems relating to the prediction of digestibility from chemical composition are discussed. Differences in chemical composition appear to exist among species of grasses, but with few exceptions the relation of chemical composition to digestibility appear to be similar in all species of grass. No distinction has been made within species of grass in these studies. The differences between grasses and alfalfa are more distinct, and the same relationships do not hold in many predictions of digestibility from chemical composition. These differences are illustrated by the fact that the coefficients of correlation are lower and the coefficients of variation are higher when a population consists of both grass samples and alfalfa samples than when the grasses and alfalfas are considered separately.

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